Rashmi M G

DNA replication

1. Introduction to DNA replication
2. Methods of replication
3. Semi conservative replication
4. Meselson and Stahl experiment
5. Taylor's experiment
6. Replicon and origin of replication
7. DNA replication in prokaryotes
8. Biochemistry of DNA replication
9. Common enzymes involved in DNA replication
10. DNA polymerase
11. Components of DNA Polymerase III
12. Steps involved in prokaryotic DNA replication
13. DNA replication in eukaryotes
14. Unique aspects of eukaryotic DNA replication
15. Eukaryotic DNA Polymerases
16. Proof reading
17. Comparison between DNA replication in Prokaryotes and Eukaryotes

DNA replication:

- Transmission of chromosomal DNA from generations to generations
- Achieved when chromosomal DNA is accurately replicated
- Pairing AΞT, G=C-important role in replication
- Providing two copies of the entire genome
- For distribution into each daughter cells

Semi conservative replication

2 strands of parental double helix of DNA separated

↓

Base sequence of each parental strand –serve as template

↓

Synthesis of new complementary strand

↓

2 identical progeny double helices

↓

Parental double helix is half conserved (each parental strand (single) remaining intact)

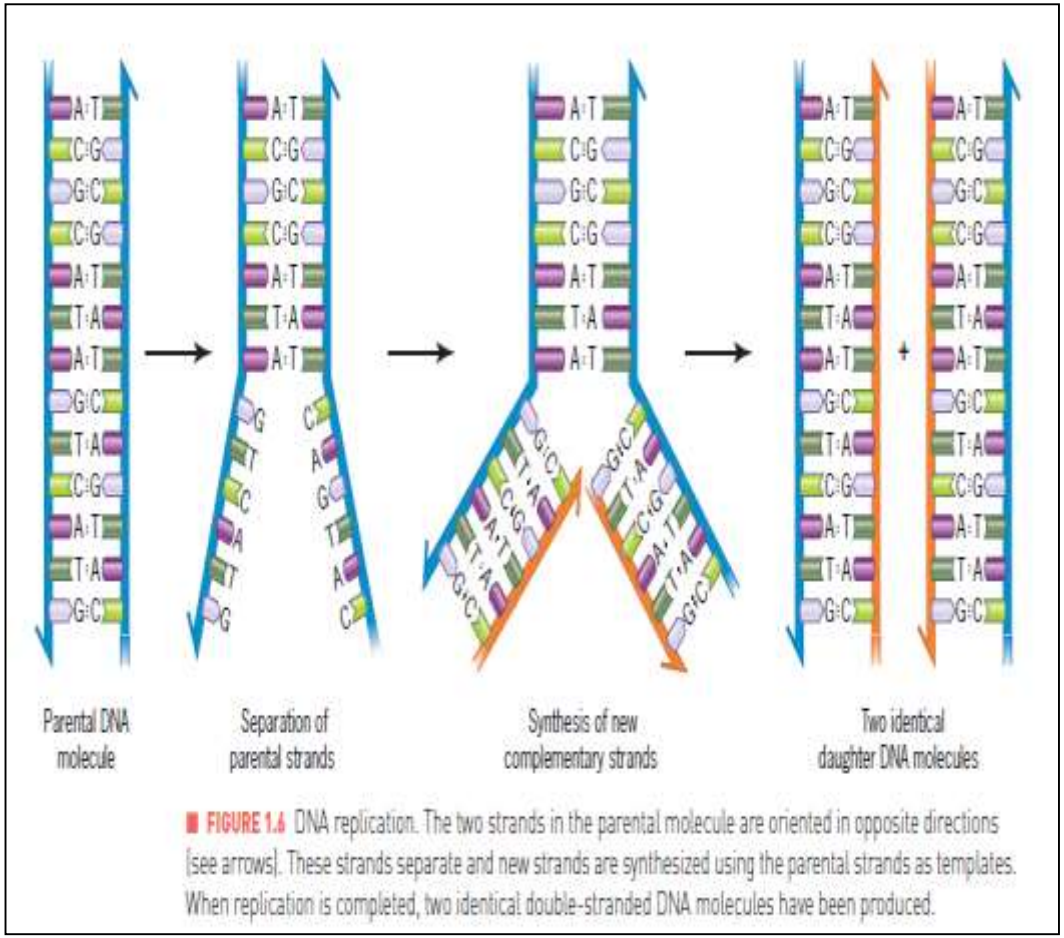

Parental DNA molecule
Separation of parental strands
Synthesis of new complementary strands
Two identical daughter DNA molecules

FIGURE 1.6 DNA replication. The two strands in the parental molecule are oriented in opposite directions (see arrows). These strands separate and new strands are synthesized using the parental strands as templates. When replication is completed, two identical double-stranded DNA molecules have been produced.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 6

Methods of replication

Conservative replication

Whole original double helix acts as template for a new one

One daughter molecule would consist of original parental DNA

2nd daughter had totally new DNA

Semi conservative replication

Parental double helix is half conserved, each parental single strand remaining intact

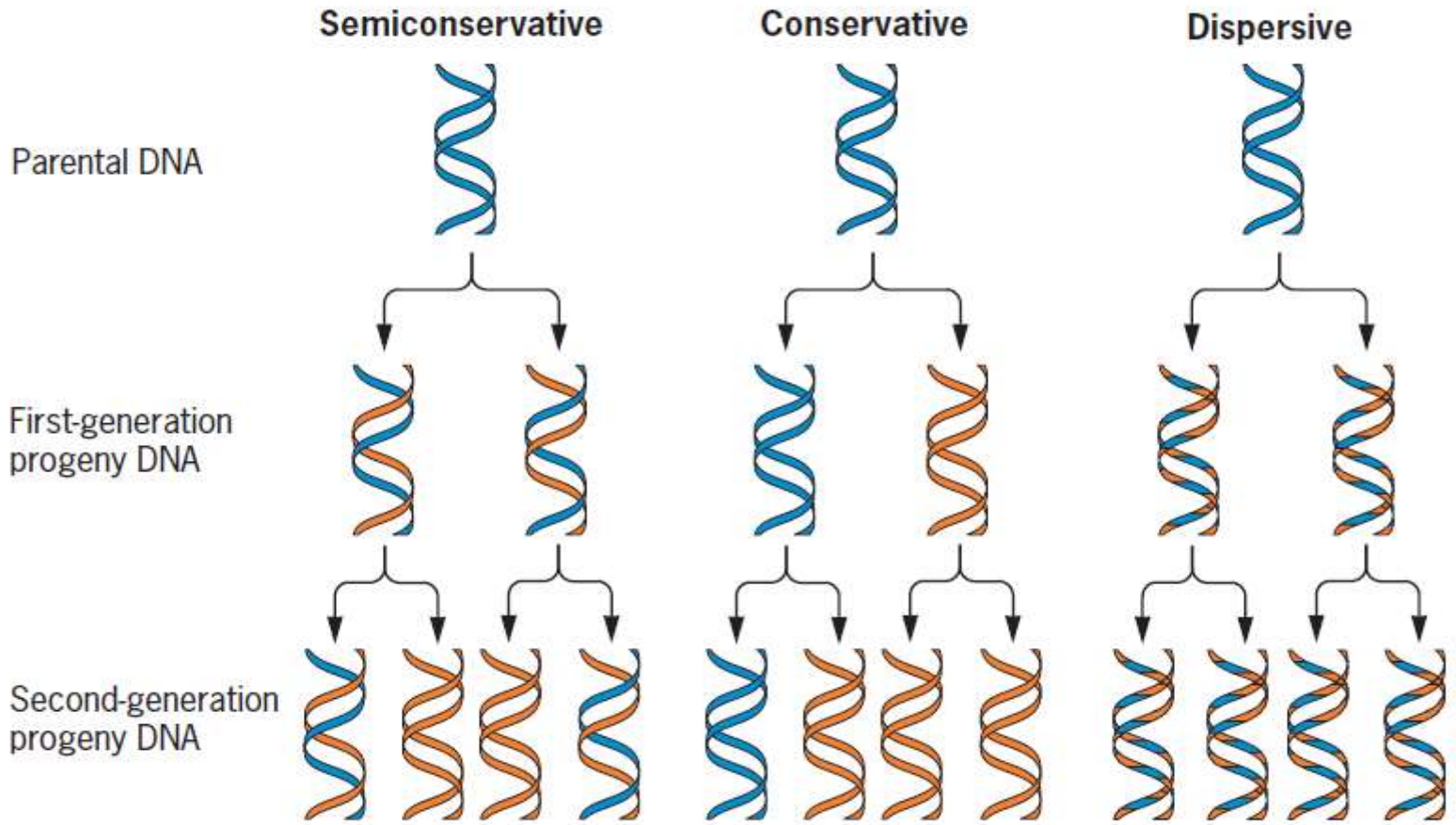
Each parental strand acts as template

Dispersive replication

Some parts of double helix are conserved and some parts are not

Parental double strand helix, broken into double stranded DNA segments

Synthesis of new double stranded DNA segment= same as conservative



■ **FIGURE 10.2** The three possible modes of DNA replication: (1) semiconservative, in which each strand of the parental double helix is conserved and directs the synthesis of a new complementary progeny strand; (2) conservative, in which the parental double helix is conserved and directs the synthesis of a new progeny double helix; and (3) dispersive, in which segments of each parental strand are conserved and direct the synthesis of new complementary strand segments that are subsequently joined to produce new progeny strands.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 222

Meselson and Stahl experiment

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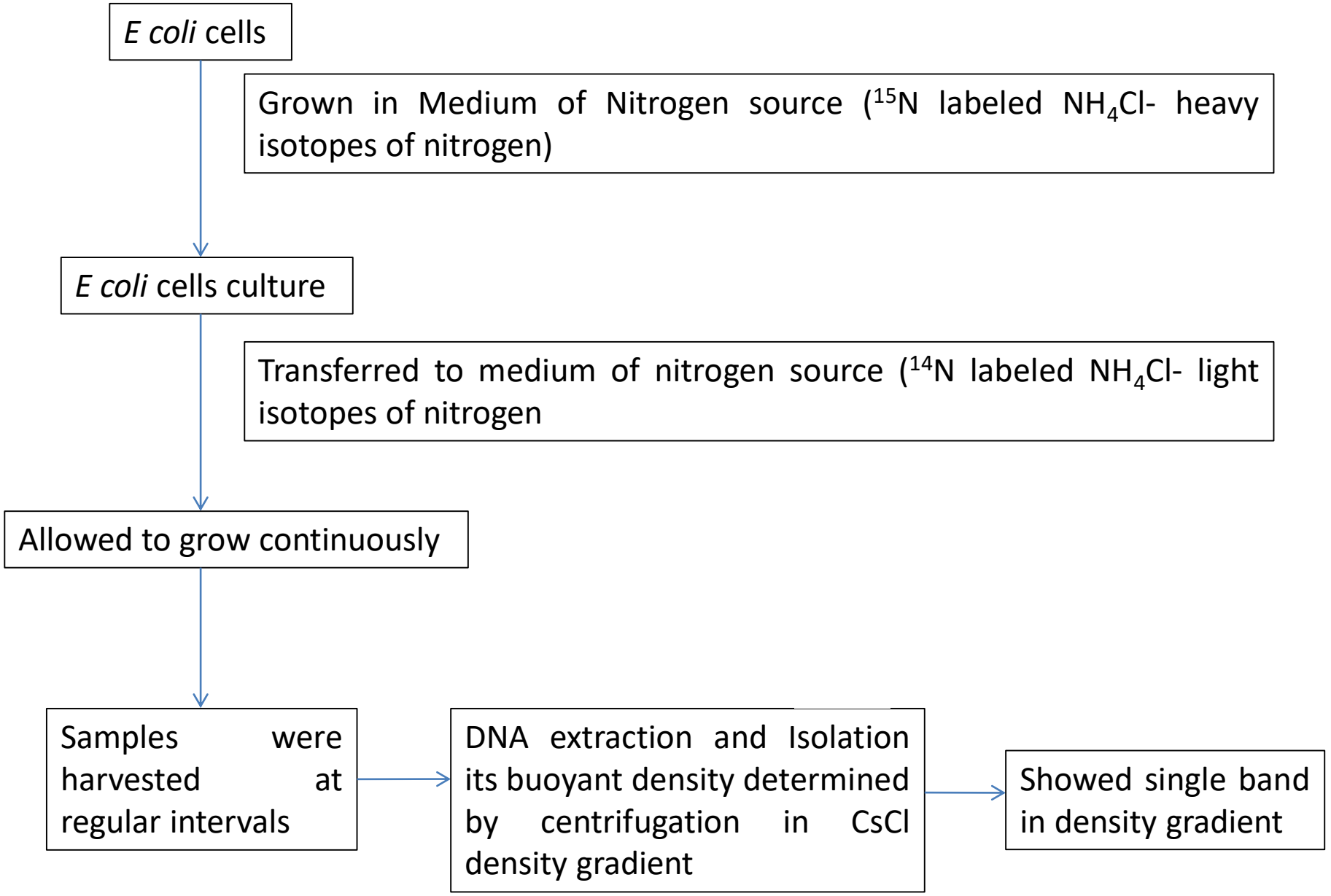
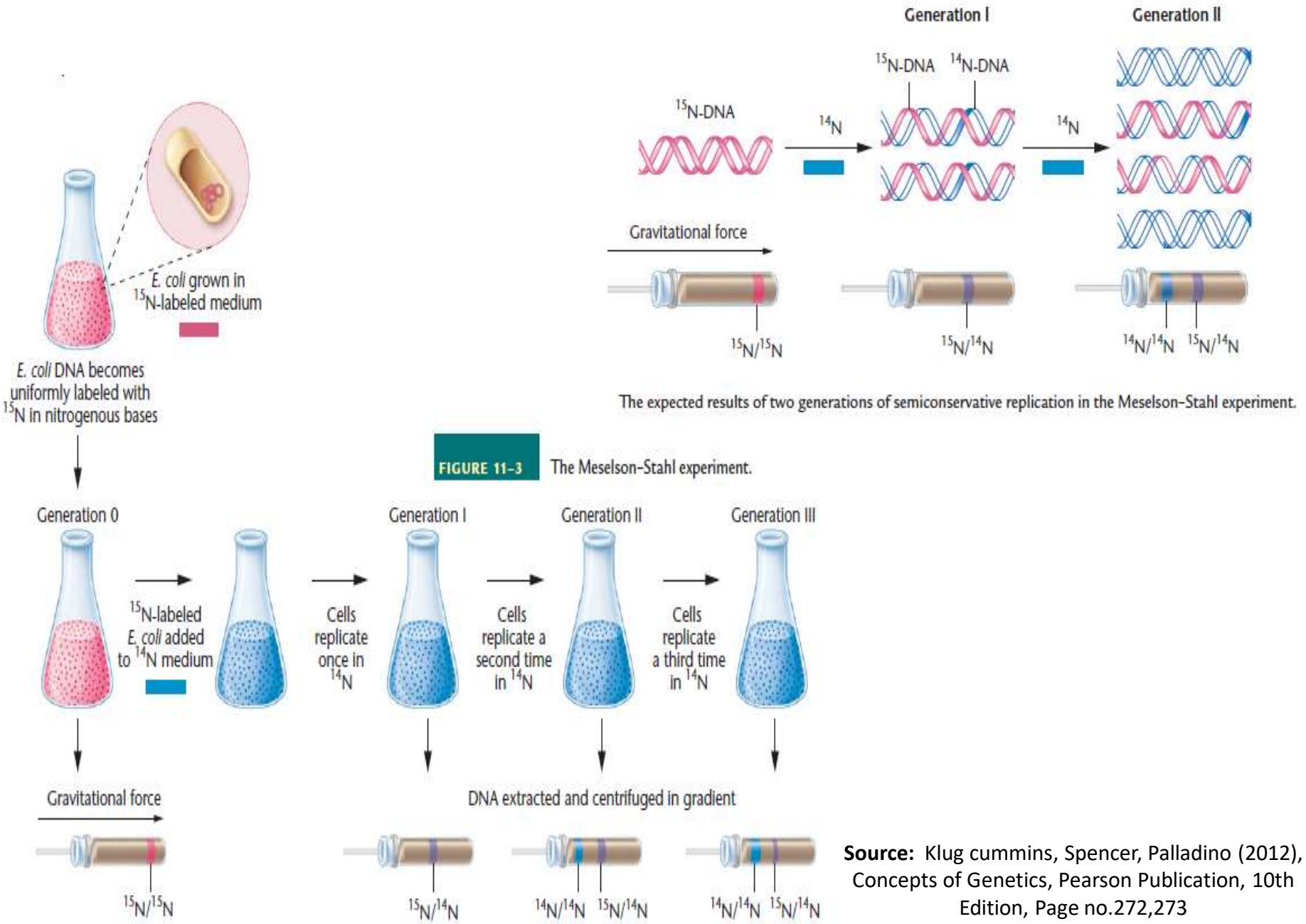
Aim : To Demonstrate of semi conservative replication of DNA in *E coli*


E coli cells
Grown in Medium of Nitrogen source (^{15}N labeled NH_4Cl- heavy isotopes of nitrogen)
E coli cells culture
Transferred to medium of nitrogen source (^{14}N labeled NH_4Cl- light isotopes of nitrogen
Allowed to grow continuously
Samples were harvested at regular intervals
DNA extraction and Isolation its buoyant density determined by centrifugation in CsCl density gradient
Showed single band in density gradient

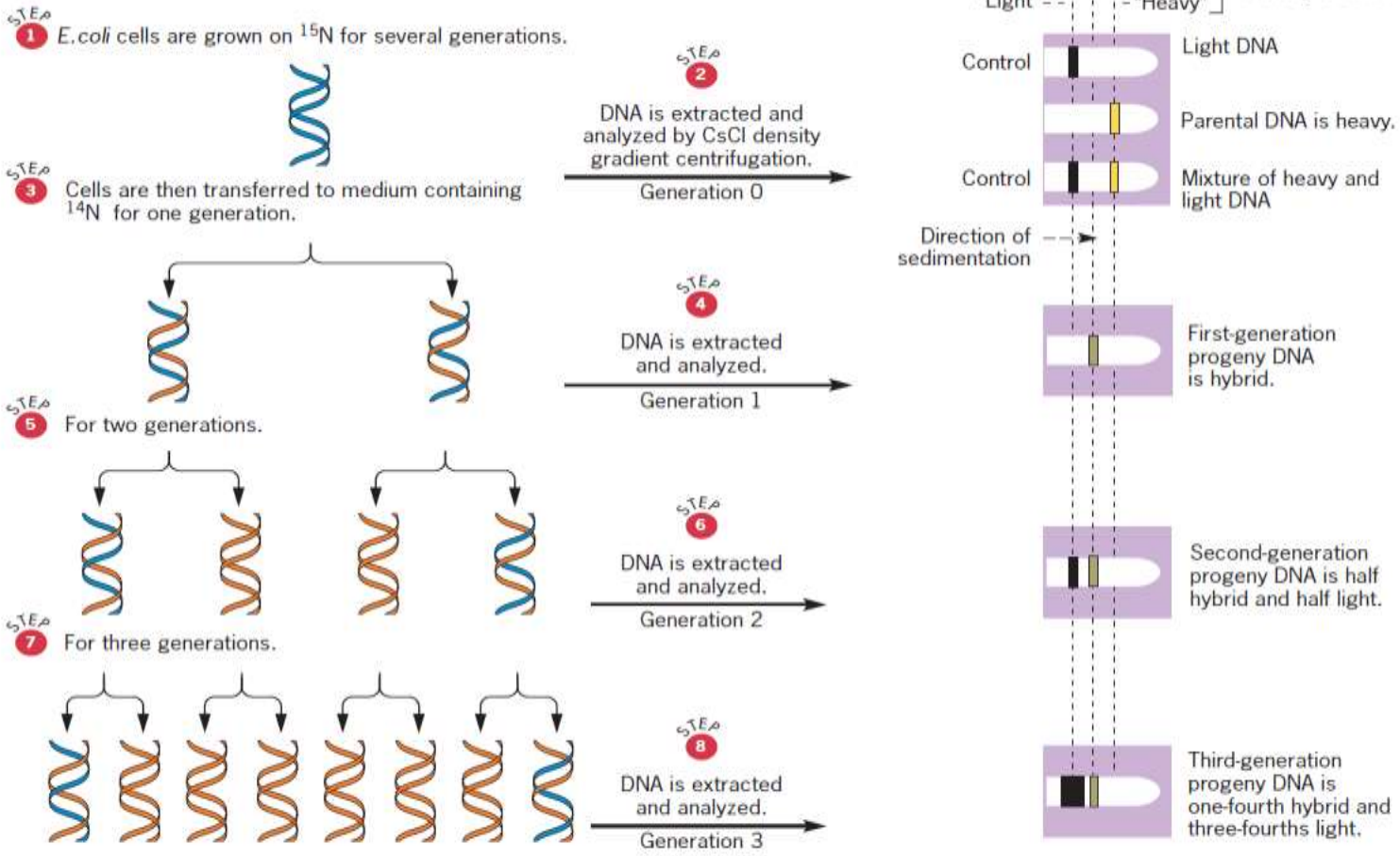


The expected results of two generations of semiconservative replication in the Meselson–Stahl experiment.

FIGURE 11–3 The Meselson–Stahl experiment.

Source: Klug cummins, Spencer, Palladino (2012), Concepts of Genetics, Pearson Publication, 10th Edition, Page no.272,273

Result:



■ **FIGURE 10.4** Meselson and Stahl's demonstration of semiconservative DNA replication in *E. coli*. The diagram shows that the results of their experiment are those expected if the *E. coli* chromosome replicates semiconservatively. Different results would have been obtained if DNA replication in *E. coli* were either conservative or dispersive (see Figure 10.2).

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 224

Conclusion: DNA replication follows Semi conservative method

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Taylor's experiment

Aim: to suggest and conclude the semi conservative method of replication with the help of *Vicia faba*

Root tips of *Vicia faba* (broad beans)

↓

Chromosomes isolated and duplicated with thymidine labeled

↓

Observed in autoradiography

↓

Allowed to duplicate without labeled thymidine

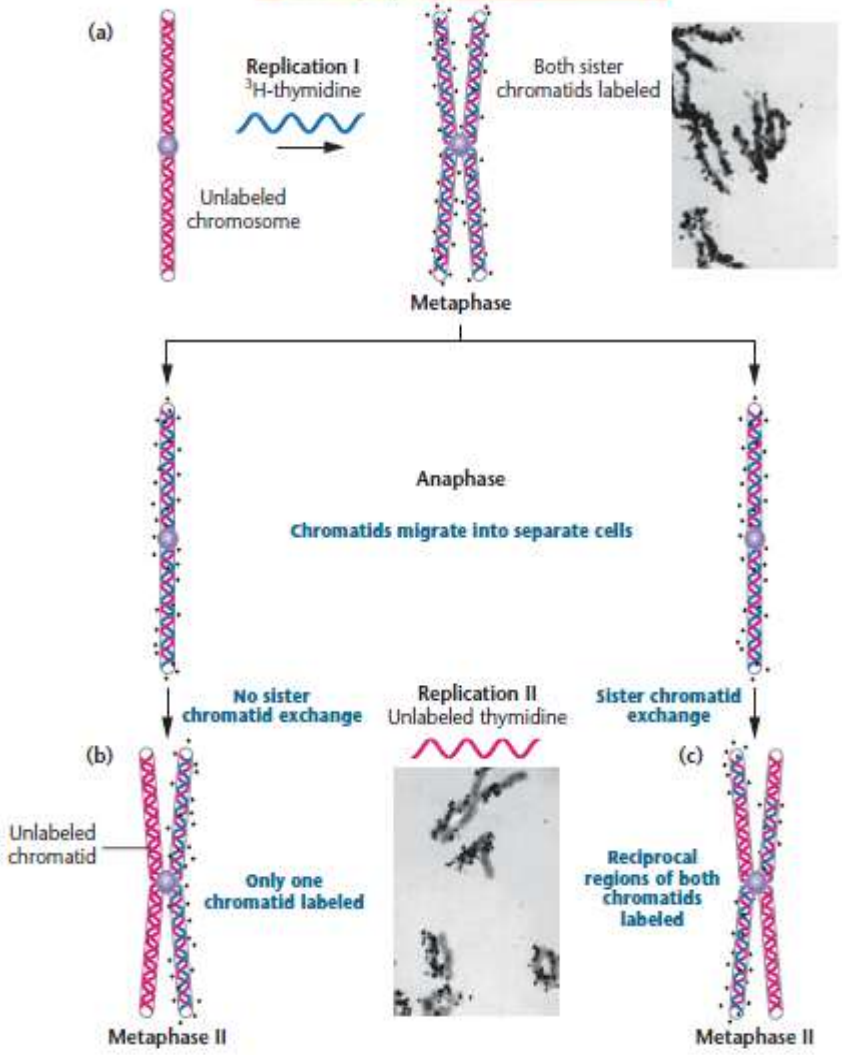
↓

Again observed in autoradiography

Conclusion:

Proves the semi conservative replication of chromosome in broad bean

Result:



Source: Klug cummins, Spencer, Palladino (2012), Concepts of Genetics, Pearson Publication, 10th Edition, Page no.273

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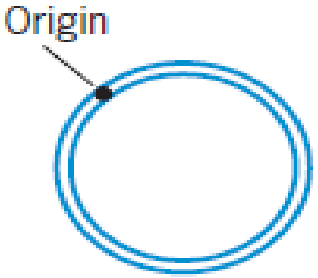
Replicon and origin of replication

- DNA replication starts at particular site- **origin of replication**
- Unit of DNA in which replication starts from an origin and proceeds bidirectionally/ unidirectionally to terminus site- **replicon** (may be linear / circular)

Replicon

Monorepliconic	**Multirepliconic**
Bacterial genome Whole bacterial genome= a single replicon Contains single replication origin	Eukaryotic genome Eukaryotic cells has multiple replication origins on single chromosomes



In prokaryotes, replicon- circular

Origin of replication

- Cis acting sequence
- Fully methylated origin
- Can initiate replication
- In *E coli*, the origin of replication is oriC
- It has 245 bp of DNA
- Contains 2 short repeat motifs (9 nucleotides and 13 nucleotides
- 9 Nucleotides repeat, 5 copies of which are dispersed throughout oriC- Binding site for a protein- Dna A
- 13 Nucleotides repeat, located at one end of the oriC Sequence

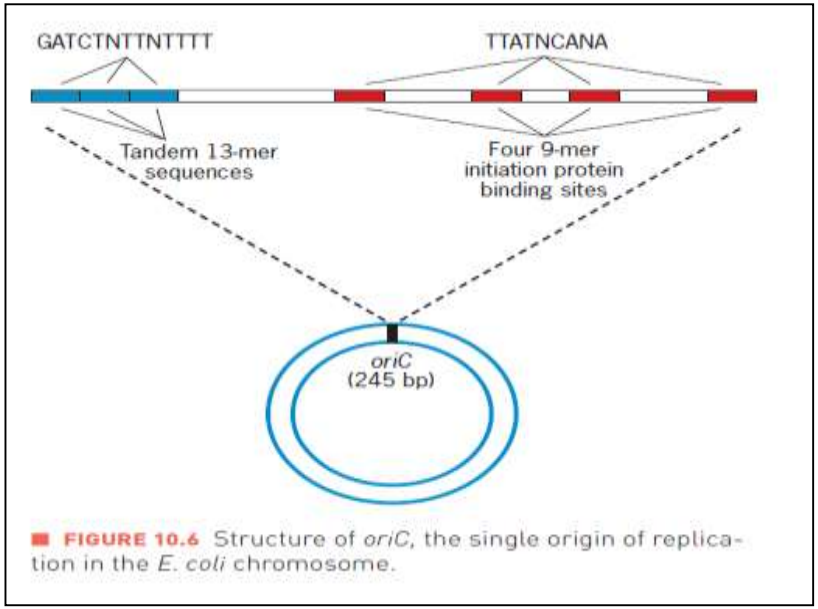


FIGURE 10.6 Structure of *oriC*, the single origin of replication in the *E. coli* chromosome.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 227

Common features of replication origins:

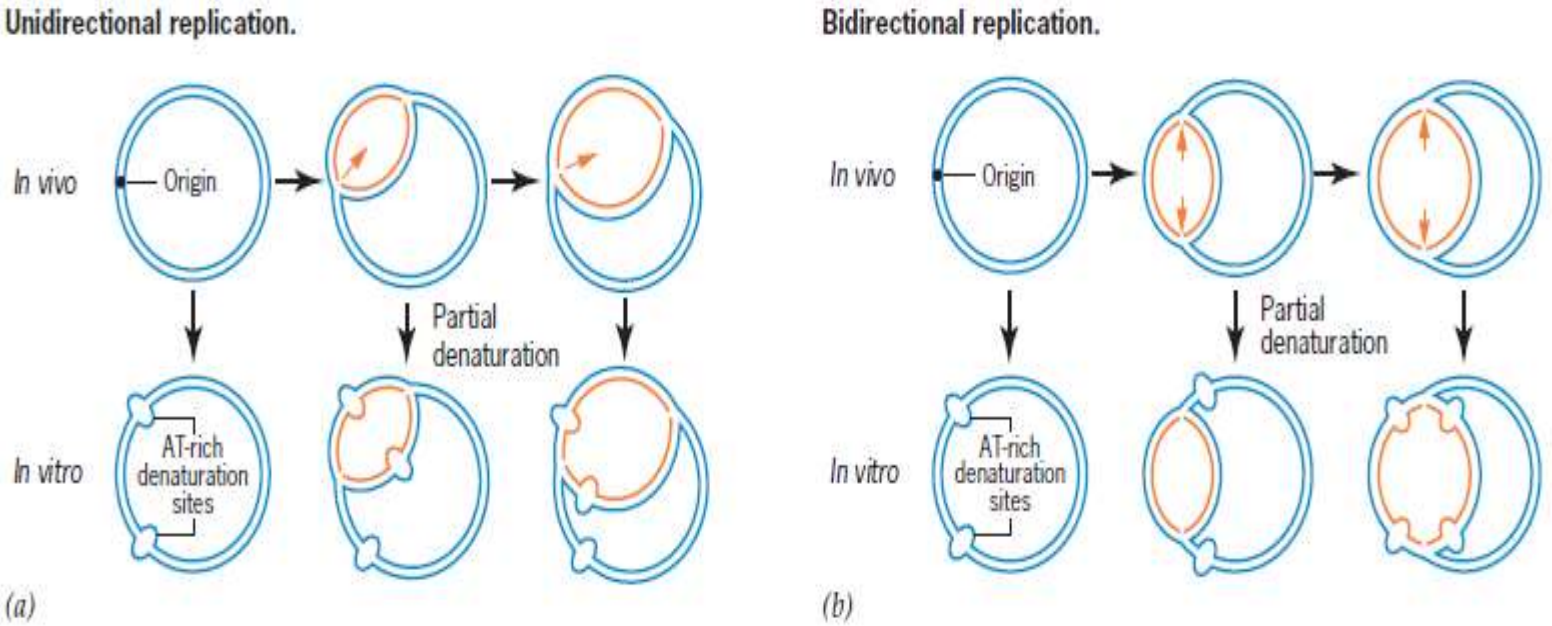
- Replication origins are unique DNA segments that contain multiple short repeated sequences
- Three short repeat units are recognized by multimeric origin binding proteins.
- These proteins play a key role in assembling DNA polymerase and other replication enzymes at the sites, where replication begins
- Origin regions usually contain AT-rich stretch. This property facilitates unwinding of duplex DNA because less energy is required to melt A-T base pairs

Replication fork

In each replicon, replication is continuous from origin to terminus
Accompanied by movement of replicating point- replication fork
Replication may be, unidirectional or bidirectional

Unidirectional replication
Single replication fork in one direction
Special types
Chloroplasts, mitochondria and Plasmids

Bidirectional replication
2 replication forks move in opposite directions
Eukaryotes (common)
Bacteria



■ **FIGURE 10.10** Rationale of the denaturation mapping procedure used by Schnös and Inman to distinguish between (*a*) unidirectional and (*b*) bidirectional modes of chromosome replication.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 229

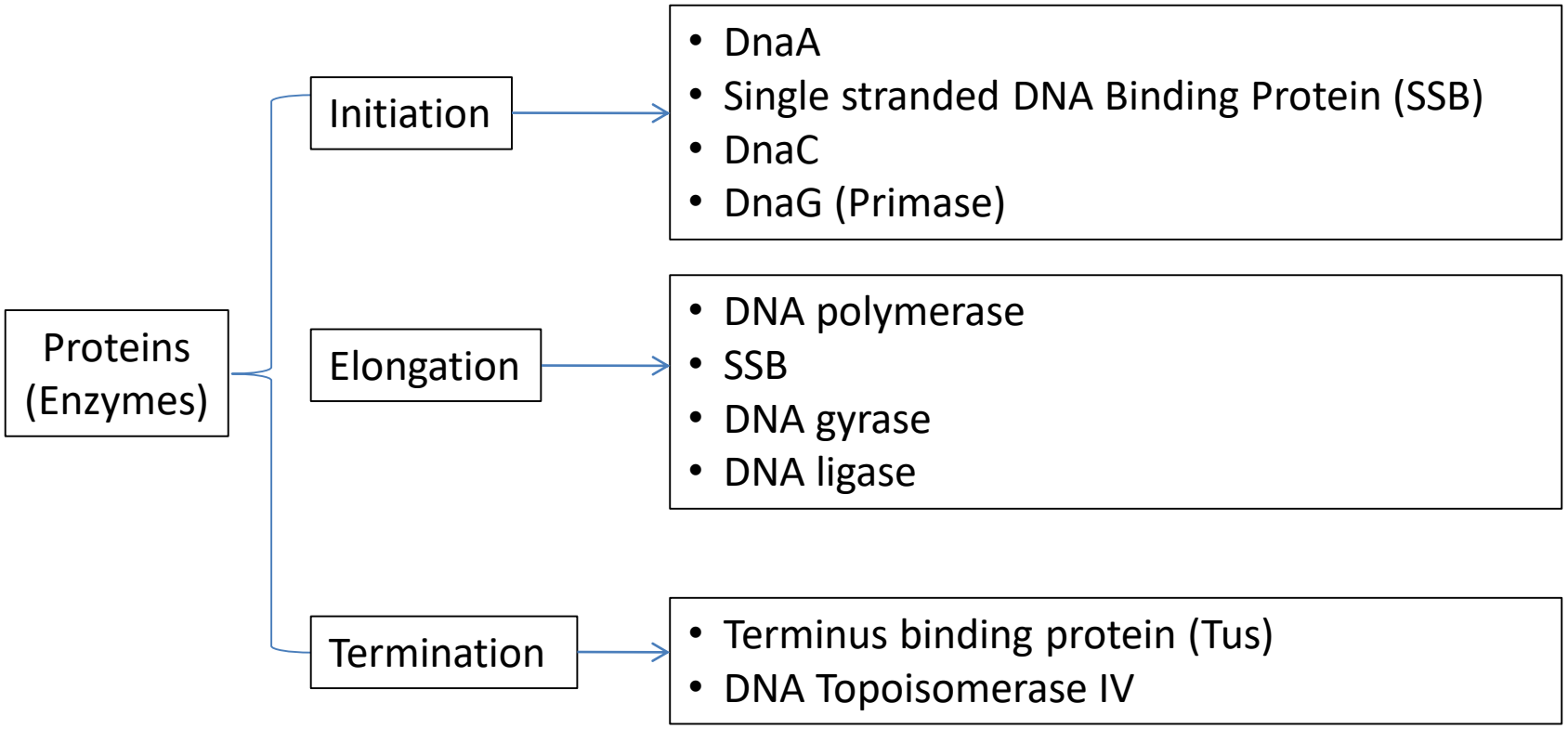
DNA replication in Prokaryotes (*E coli*)

DNA replication in *E coli*:

- Bidirectional replication
- From a single origin –OriC
- Replicon- circular with no free ends
- Replication of DNA in *E coli*= Theta replication
- 3 steps:
- **Initiation** – recognition of the position on a DNA molecule where replication will begin
- **Elongation-** events occurring at the replication fork, where parent polynucleotides are copied
- **Termination** – occurs when parent molecule has been completely replicated

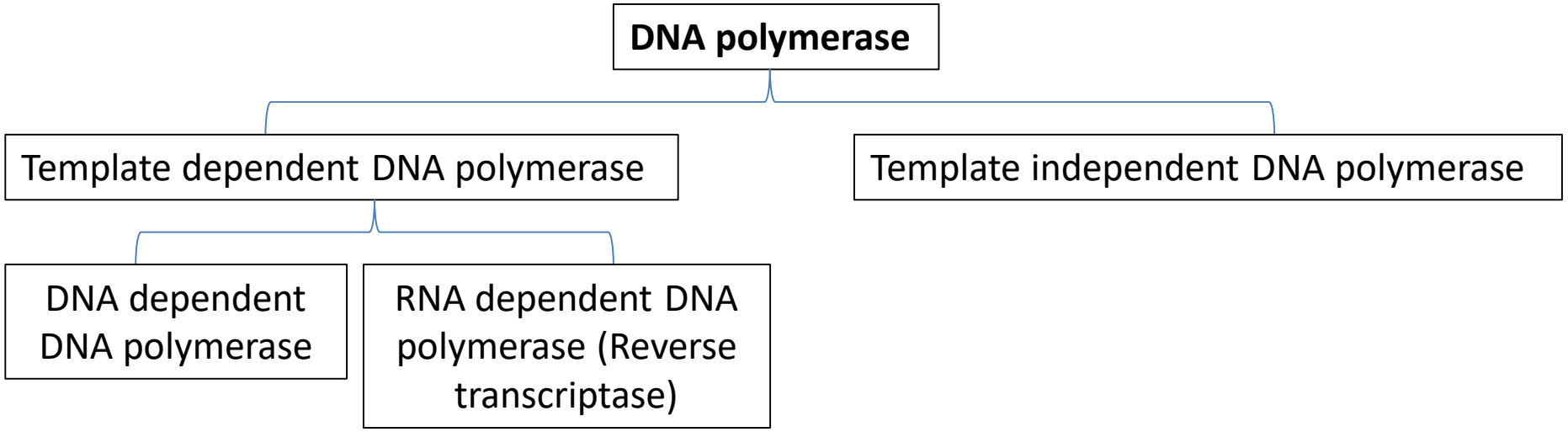
Biochemistry of DNA replication:

In *E coli* >20 proteins take part in replication



Common enzymes and proteins involved:

<table>
<tr>
<td>DNA helicase and primase
• Opens up the duplex at the replication fork to provide a single stranded template
• Primary replicative helicase- DnaB – binds to and moves on the lagging strand template in the 5’→3’ direction unwinding the duplex as it goes
• Requires ATP hydrolysis</td>
<td>SSB Protein
• Inhibition of reannealing of separated strands
• Bindings to both separated strands</td>
<td>Primase
• Synthesizes a short RNA primer (<15 nucleotides) to prime DNA chain elongation
• Primosome- complex between primase and helicase, sometimes with accessory proteins</td>
</tr>
<tr>
<td>Topoisomerase
• Required to relieve the positive super coiling that arises from DNA unwinding
• In E coli this role is typically fulfilled by DNA gyrase
• In eukaryotes TopoIB</td>
<td colspan="2">DNA Polymerase
• Catalyze the synthesis of DNA
• Both prokaryotes and Eukaryotes contain multiple DNA dependent DNA polymerase
• Termed as DNA Replicase
• Performs DNA repair also</td>
</tr>
</table>


DNA polymerase
Template dependent DNA polymerase
Template independent DNA polymerase
DNA dependent DNA polymerase
RNA dependent DNA polymerase (Reverse transcriptase)

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In Eubacteria, - *E coli*

5 types of DNA polymerases

DNA polymerase I

- Kornberg enzyme
- Arthur Kornberg enzyme
- Monomeric protein
- Possess 3 enzymatic activities:
- 5'→3' Polymerase activity
- 5'→3' exonuclease activity
- 3'→5' exonuclease activity
- Primer removal function (with 5'→3' exonuclease activity)
- Gap filling (5'→3' polymerase activity) and DNA repair

DNA polymerase II

- Monomeric protein
- 5'→3' Polymerase activity
- 3'→5' exonuclease activity
- Alternative DNA repair polymerase
- Can replicate DNA if the template is damaged
- Low polymerizatio n rate

DNA polymerase III

- Primary enzyme
- Multiprotein complex
- (10 distinct polypeptides)
- High polymerization rate
- High Processivity

DNA polymerase IV

- Y family DNA polymerase
- dinB
- Do not contain 3'→5' exonuclease activity
- Low catalytic efficiency
- Low Processivity
- Low fidelity
- Involved in tranlesion synthesis
- Replicate damaged DNA by bypassing damaged nucleotides

DNA polymerase V

- Y family DNA polymerase
- Found in bacteria like *E coli*
- Error prone polymerase
- Involved in SOS response to extensive DNA damage

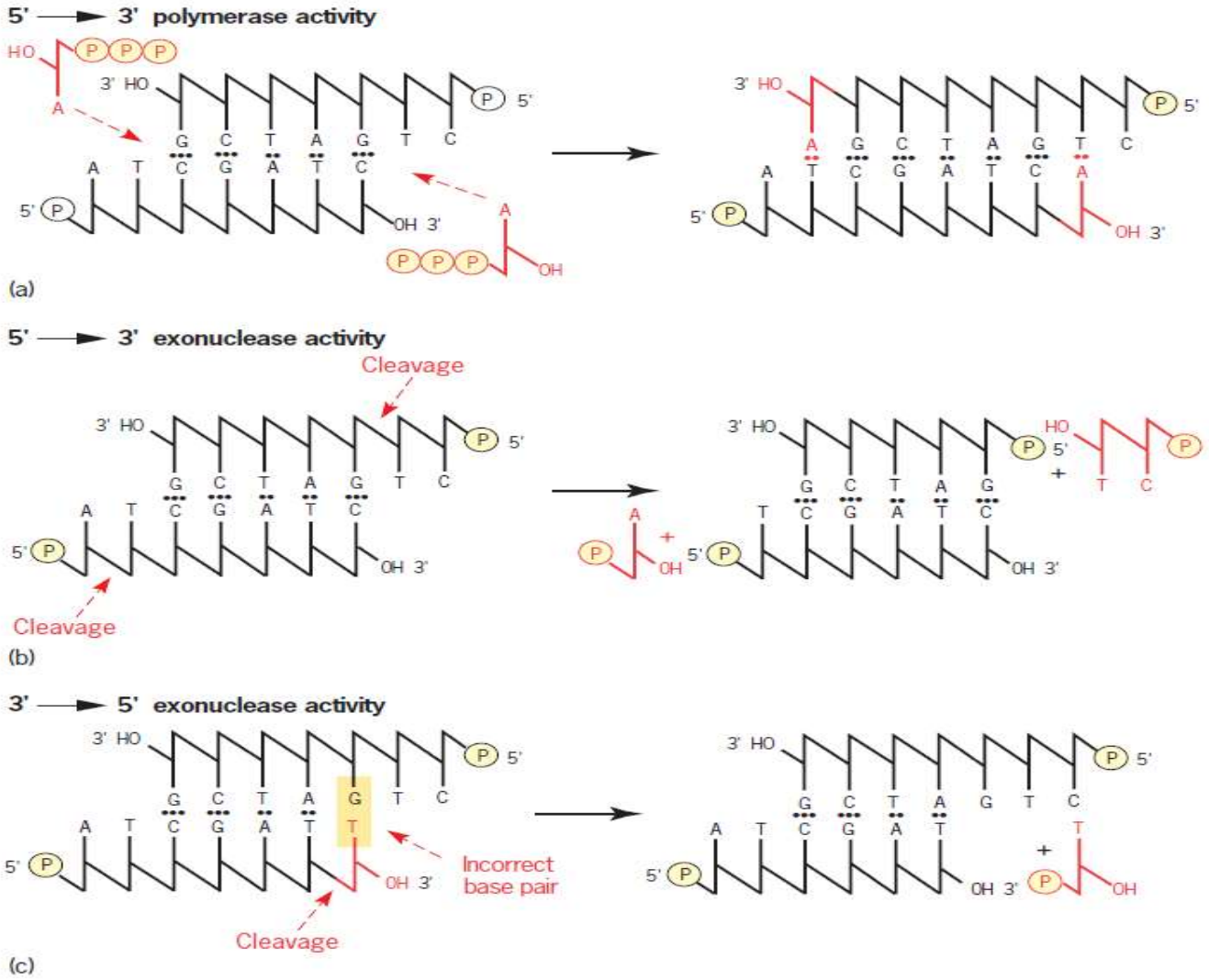

FIGURE 10.17 The three activities of DNA polymerase I in *E. coli*. The DNA molecules are shown here using flattened "stick" diagrams with one complementary strand on the top and the other on the bottom. "Stick" diagrams nicely emphasize the opposite chemical polarity (5′ → 3′ and 3′ → 5′) of the complementary strands. As is discussed in the text, all three activities—(*a*) 5′ → 3′ polymerase activity, (*b*) 5′ → 3′ exonuclease activity, and (*c*) 3′ → 5′ exonuclease activity—play important roles in *E. coli* cells.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 235

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Components of DNA Polymerase III

Catalytic core

- α
- β
- θ

Dimerization component

τ

Causes 2 catalytic core at replication fork to link together and form asymmetrical dimer

Processivity component

Homodimer of β subunits (acts as sliding clamp)

Clamp loader

5 subunits subassembly

γ | δ | χ | ψ | $\delta_э$

ϒ complex

Loading of β clamp onto the DNA
Facilitated by ϒ complex

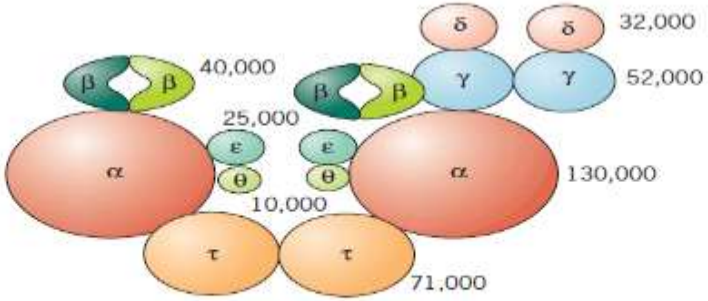

FIGURE 10.26 Structure of the *E. coli* DNA polymerase III holoenzyme. The numbers give the masses of the subunits in daltons.

- To enhance proofreading activity of ϵ
- 3'→5' exonuclease activity (Proof reading)
- 5'→3' Polymerase activity

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 241

ENZYME	EXONUCLEASE ACTIVITIES			
	Subunits	3'→5'	5'→3'	Function
DNA polymerase I	1	YES	YES	DNA repair Gap filling Primer removal
DNA polymerase II	1	YES	NO	DNA repair
DNA polymerase III	10	YES	NO	Main replicating enzyme

Major DNA polymerase involved in replication of bacterial genome

Steps involved in prokaryotic DNA replication

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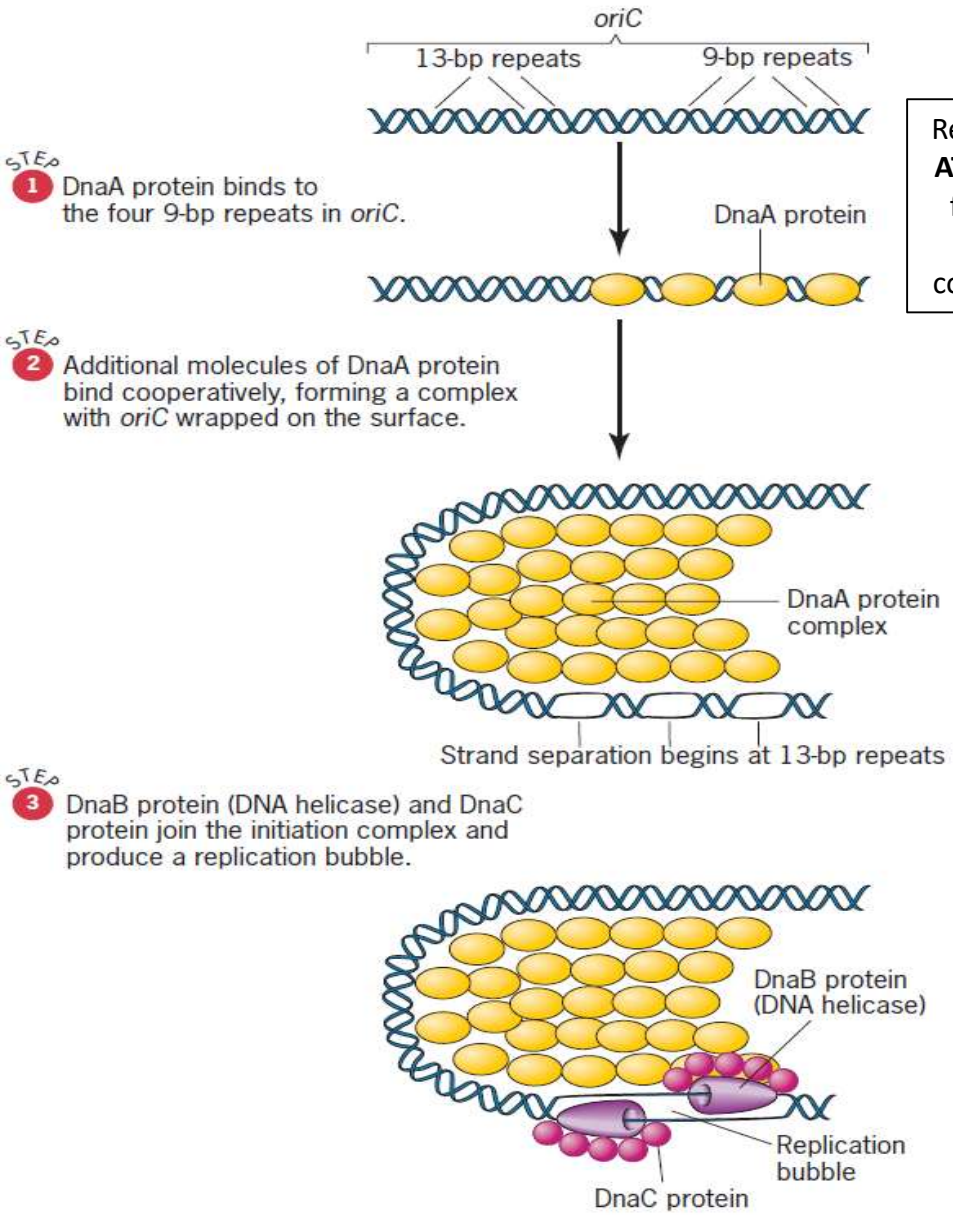
Initiation step: DnaA protein- initiates replication in *E coli* at oriC


oriC
13-bp repeats
9-bp repeats
STEP 1
DnaA protein binds to the four 9-bp repeats in oriC.
DnaA protein
STEP 2
Additional molecules of DnaA protein bind cooperatively, forming a complex with oriC wrapped on the surface.
DnaA protein complex
Strand separation begins at 13-bp repeats
STEP 3
DnaB protein (DNA helicase) and DnaC protein join the initiation complex and produce a replication bubble.
DnaB protein (DNA helicase)
Replication bubble
DnaC protein

■ **FIGURE 10.15** Prepriming of DNA replication at *oriC* in the *E. coli* chromosome.

Requires **ATP** and forms open complex

DnaA protein

+ **oriC** (9 mer sequence)

=**Initial complex** (facilitates initial strand separation or melting) Occurs at **oriC** 13 mers

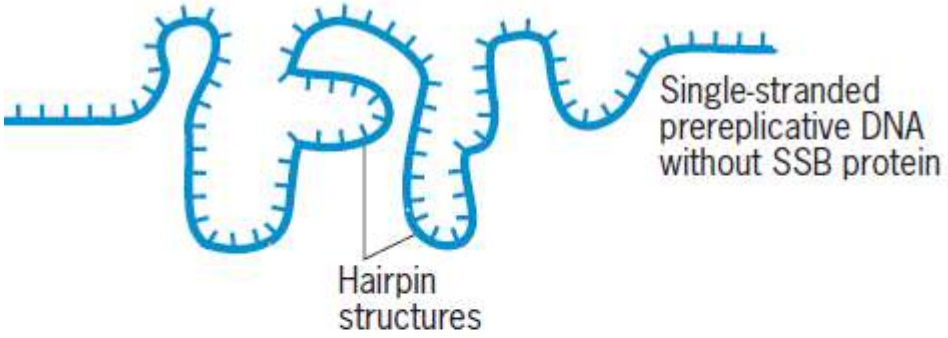
Melting of 2 strands generates unpaired template strands

Mediated by **DnaB** protein- **Helicase**

Requires **ATP** and **DnaC** acts as helicase loader

1 molecule= hexamer of identical subunit Clamp around each 2 single strand in open complex formed between **DnaA** and **oriC**

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 234



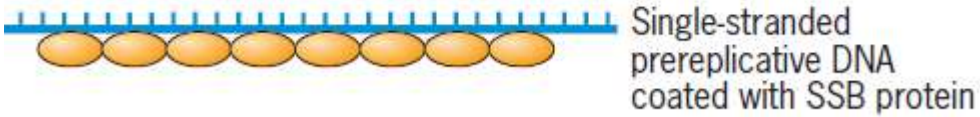


Image showing the importance of SSB protein

Helicase

Move along DNA duplex

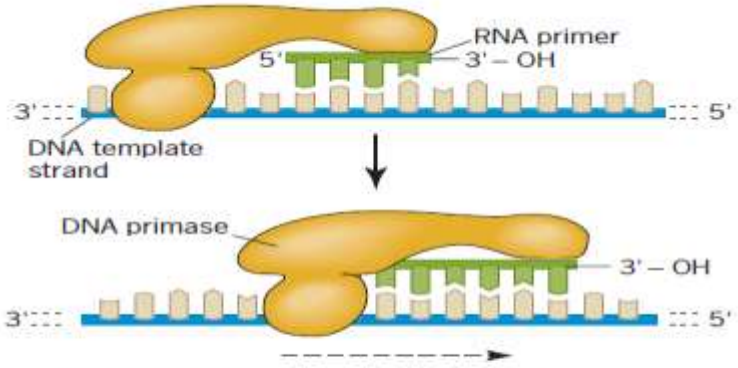
Utilize energy of ATP hydrolysis to separate strands

SSB Protein

Inhibits reannealing by binding to both separated strands

Primase

Synthesize short primer RNA complementary to both strands of DNA duplex



■ **FIGURE 10.16** The initiation of DNA strands with RNA primers. The enzyme DNA primase catalyzes the synthesis of short (10 to 60 nucleotides long) RNA strands that are complementary to the template strands.

Elongation step:

DNA polymerase catalyze step by step addition of deoxyribonucleotide units to DNA chain

Elongation reaction:

Nucleophilic attack by 3' hydroxyl group of primer on innermost phosphorus atom of deoxyribonucleoside triphosphate

Phosphodiester bridge forms= release of pyrophosphate

Catalytic metal ions (Mg^{2+}) present in active site has important role

Metal ions+ 3'OH = Reduce association between O and H

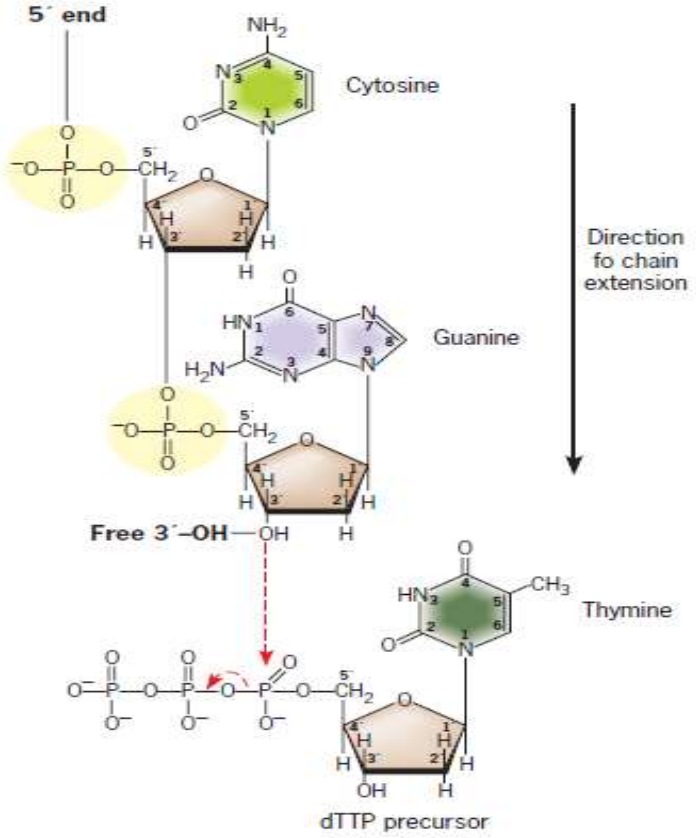
Metal ions + Triphosphate of incoming dNTP Neutralize their negative charge

leaves a nucleophilic 3'O^-

After catalysis pyrophosphate product stabilized through similar interaction with metal ions

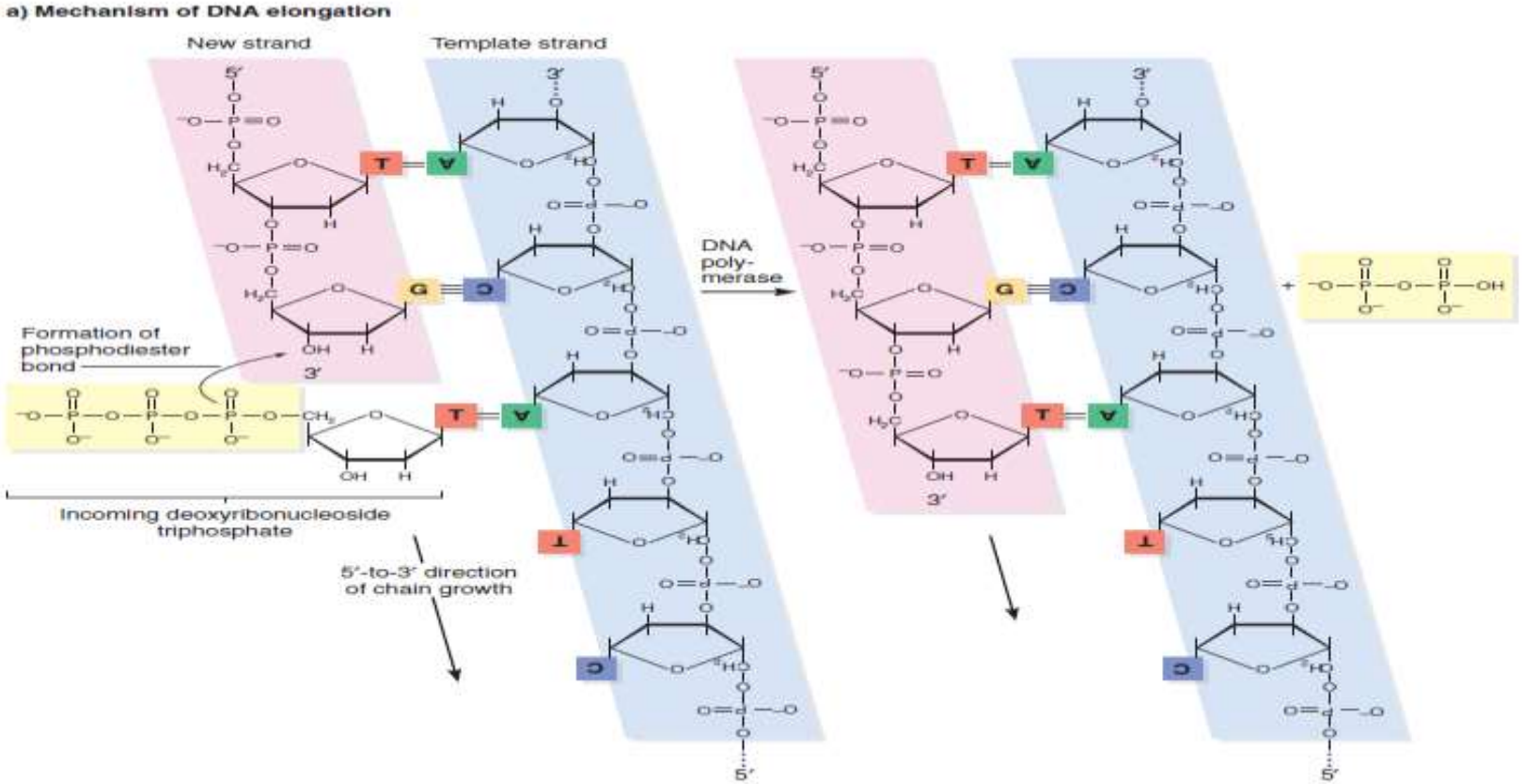
Hydrolysis by pyrophosphatase

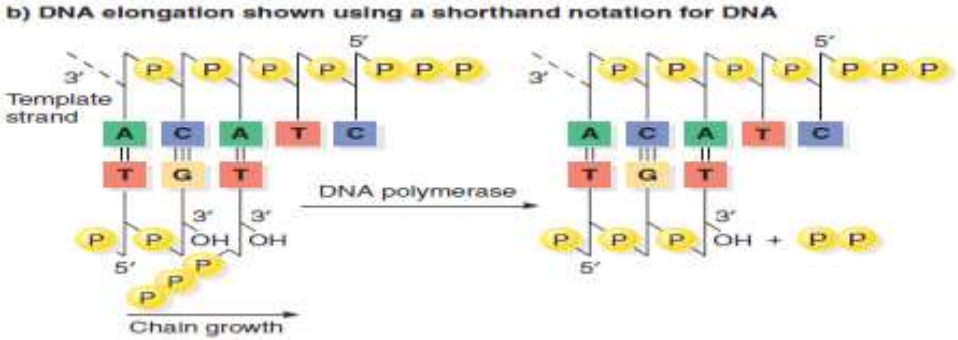
Helps to drive polymerization forward



■ **FIGURE 10.12** Mechanism of action of DNA polymerases: covalent extension of a DNA primer strand in the 5′ → 3′ direction. The existing chain terminates at the 3′ end with the nucleotide deoxyguanylate (deoxyguanosine-5′-phosphate). The diagram shows the DNA polymerase-catalyzed addition of deoxythymidine monophosphate (from the precursor deoxythymidine triphosphate, dTTP) to the 3′ end of the chain with the release of pyrophosphate (P_2O_7).

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 232





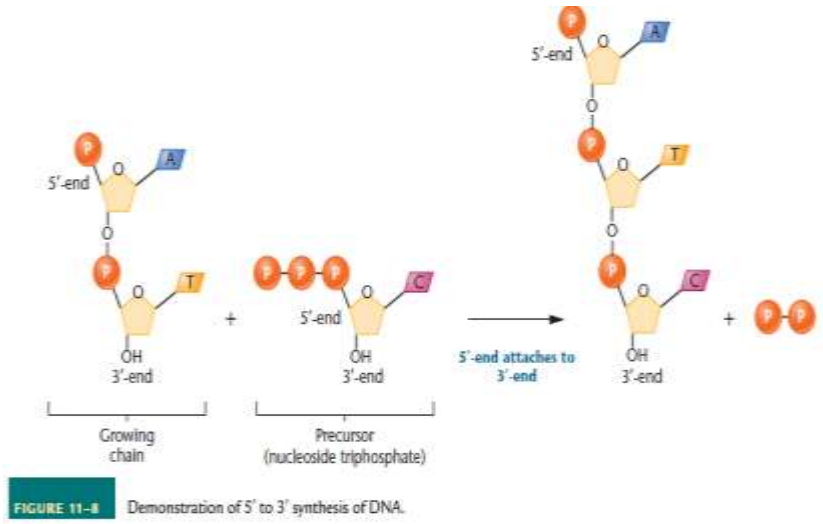
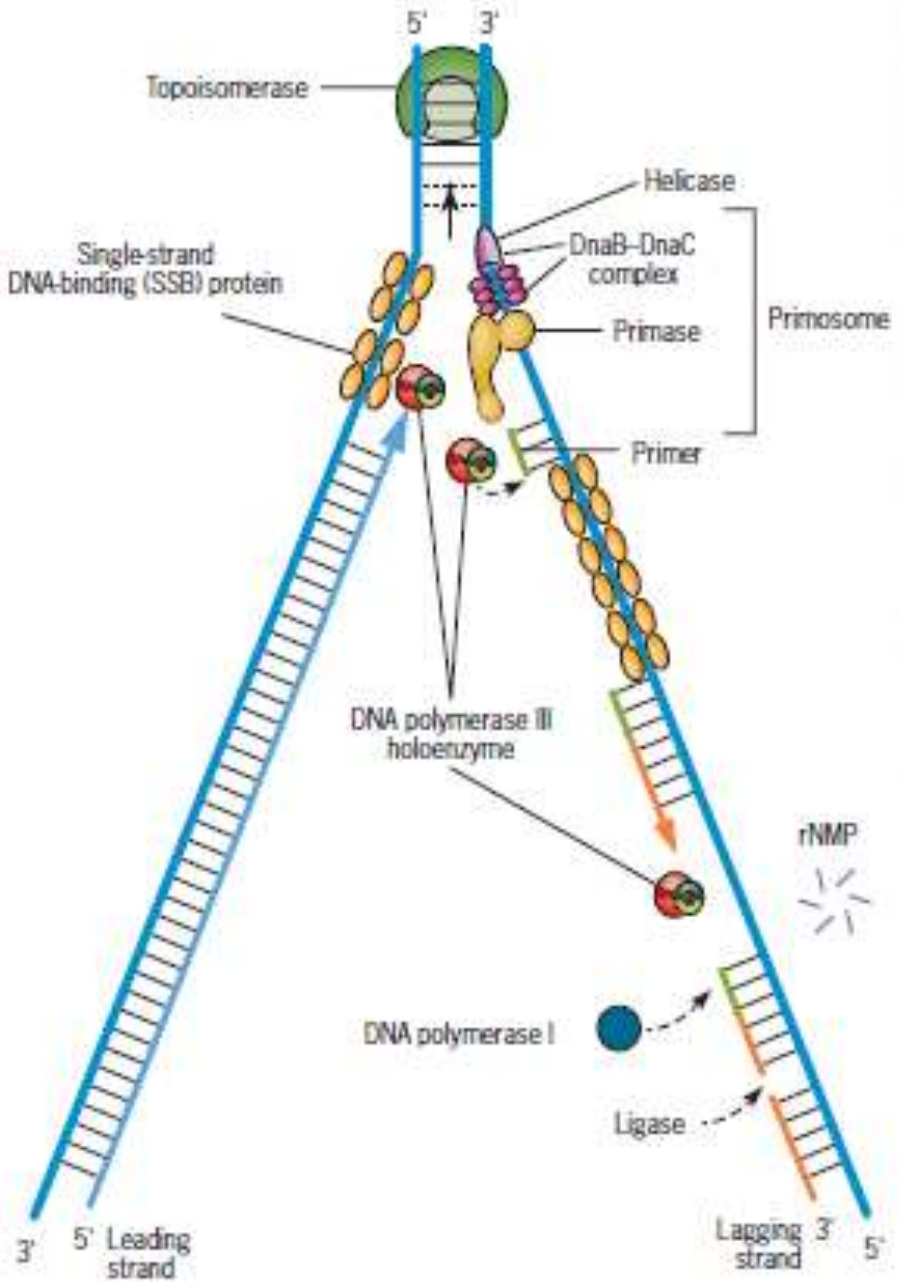


FIGURE 11–8 Demonstration of 5′ to 3′ synthesis of DNA.

Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.41

Source: Klug cummins, Spencer, Palladino (2012), Concepts of Genetics, Pearson Publication, 10th Edition, Page no.275



■ FIGURE 10.20 Diagram of a replication fork in *E. coli* showing the major components of the replication apparatus. rNMP = ribonucleoside monophosphates.

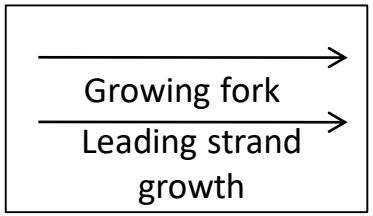
At each growing fork,

1.Leading strand:

Synthesized continuously from single primer on leading strand template

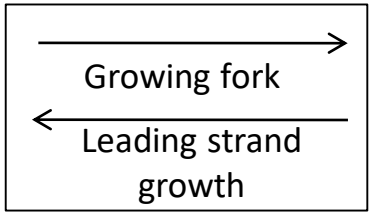
Growing fork

Grows 5'→3'



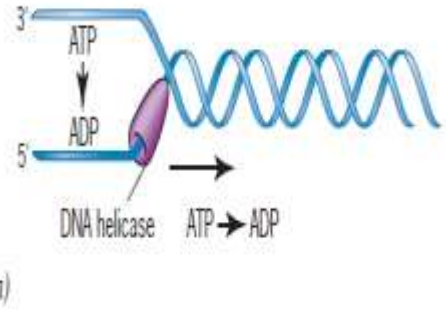
2.Lagging strand:

Complicated, DNA polymerase can add nucleotide only to 3' end of primer / growing DNA strand



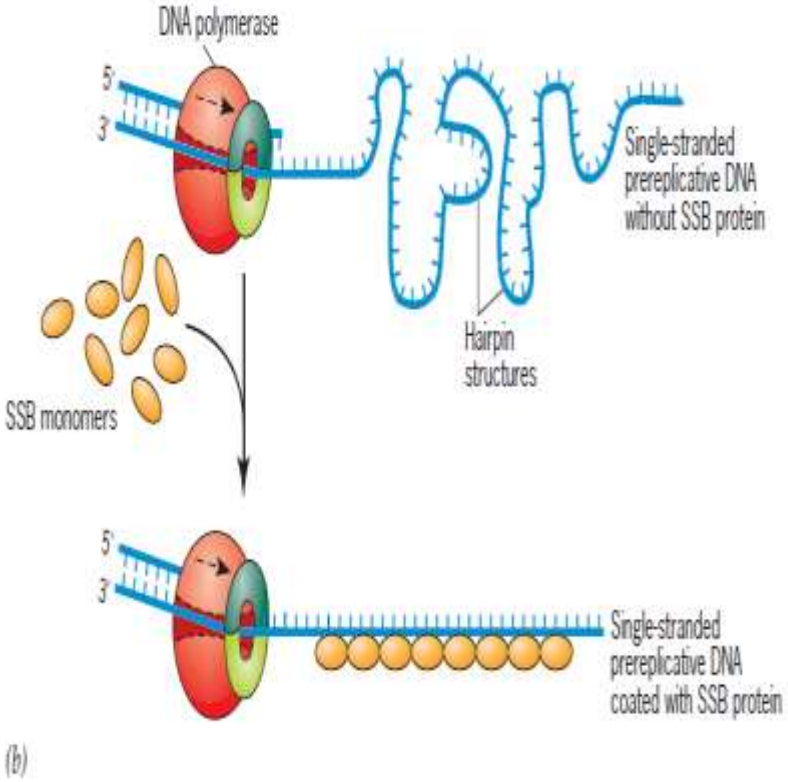
Short pieces of DNA= **Okazaki fragments** – repeatedly synthesized on lagging strand template

DNA helicase catalyzes the unwinding of the parental double helix.



(a)

Single-strand DNA-binding (SSB) protein keeps the unwound strands in an extended form for replication.



(b)

FIGURE 10.19 The formation of functional template DNA requires (a) DNA helicase, which unwinds the parental double helix, and (b) single-strand DNA-binding (SSB) protein, which keeps the unwound DNA strands in an extended form. In the absence of SSB protein, DNA single strands can form hairpin structures by intrastrand base-pairing (b, top), and the hairpin structures will retard or arrest DNA synthesis.

Primase+ helicase = Primosome

Makes RNA primer

DNA Polymerase III (synthesizing leading strand copy) extends RNA primer

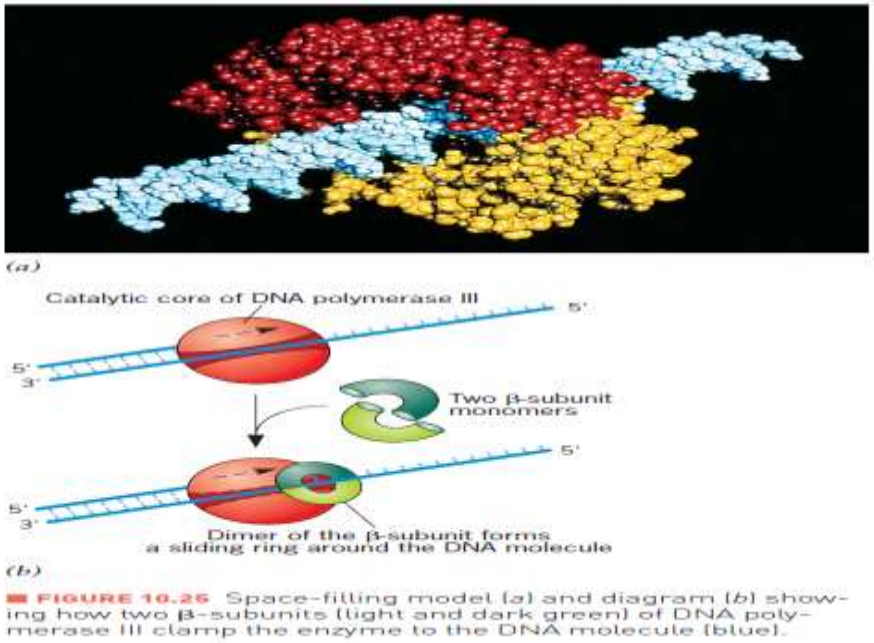
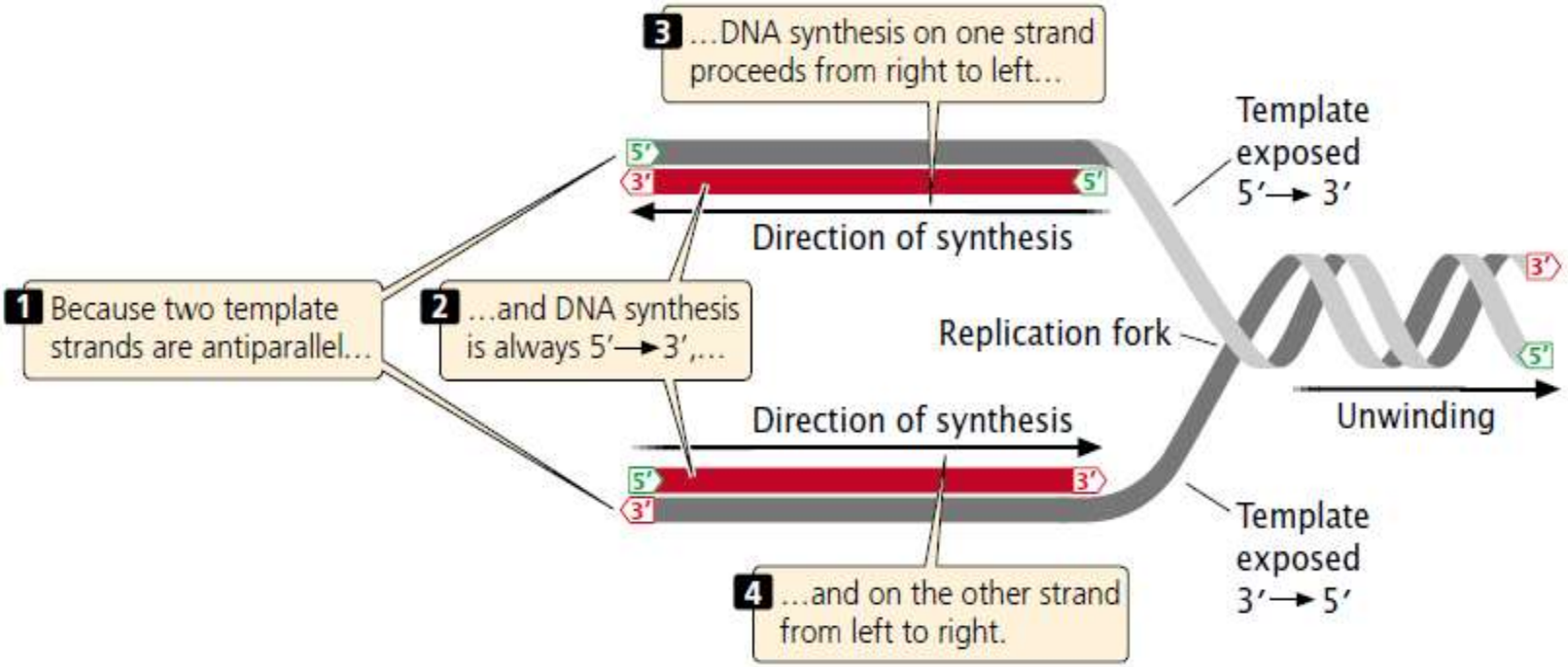


FIGURE 10.25 Space-filling model (a) and diagram (b) showing how two β-subunits (light and dark green) of DNA polymerase III clamp the enzyme to the DNA molecule (blue).

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 237

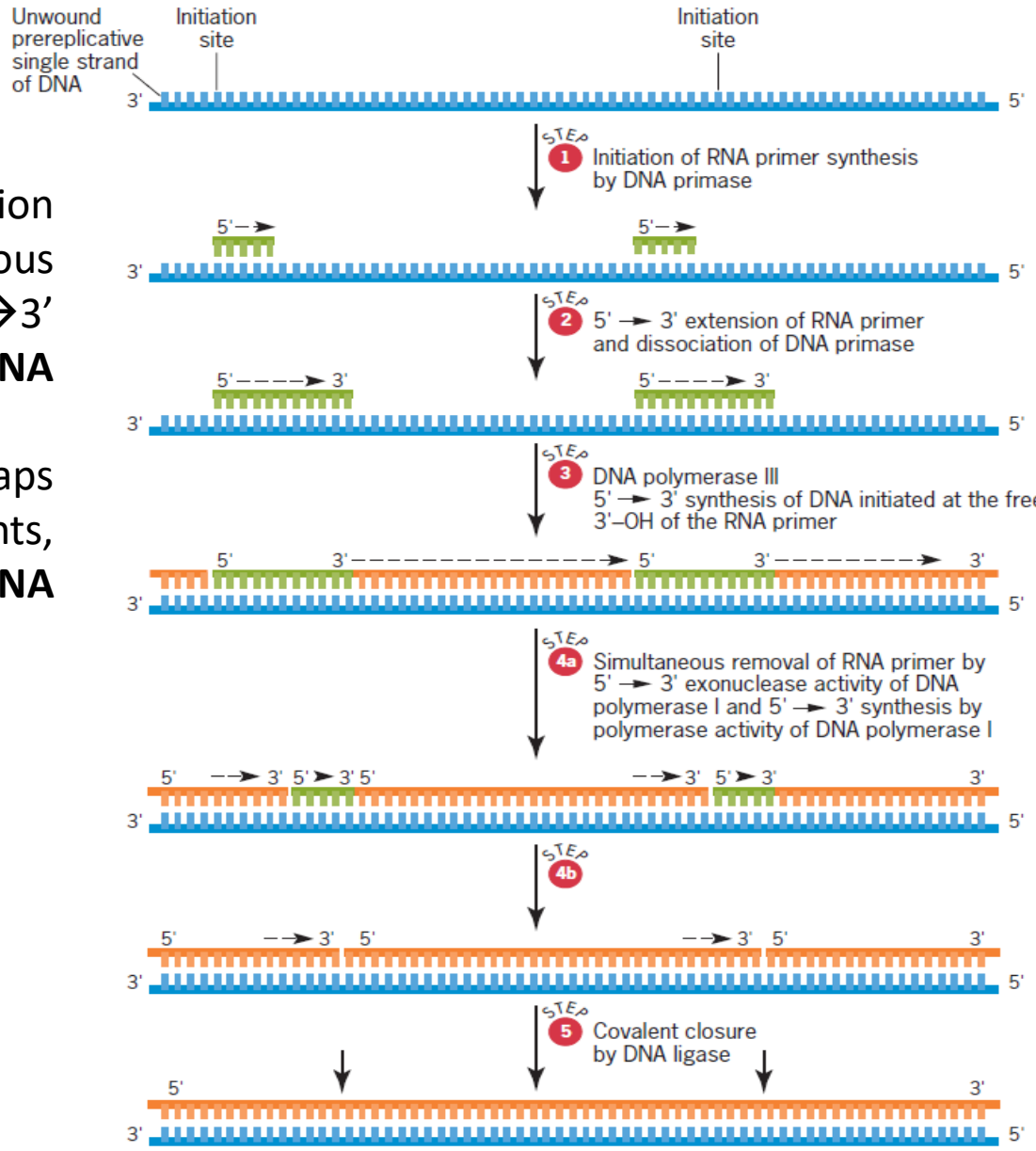


12.8 DNA synthesis takes place simultaneously but in opposite directions on the two DNA template strands. DNA replication at a single replication fork begins when a double-stranded DNA molecule unwinds to provide two single-strand templates.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 331

As each Okazaki fragment formation completes, the RNA primer of previous fragment is removed by the 5'→3' exonuclease activity of **DNA polymerase I**

This enzyme also fills in the gaps between the lagging strand fragments, which then are ligated together by **DNA ligase**


Unwound prereplicative single strand of DNA
Initiation site
Initiation site
STEP 1
Initiation of RNA primer synthesis by DNA primase
STEP 2
5' → 3' extension of RNA primer and dissociation of DNA primase
STEP 3
DNA polymerase III 5' → 3' synthesis of DNA initiated at the free 3'–OH of the RNA primer
STEP 4a
Simultaneous removal of RNA primer by 5' → 3' exonuclease activity of DNA polymerase I and 5' → 3' synthesis by polymerase activity of DNA polymerase I
STEP 4b
STEP 5
Covalent closure by DNA ligase

The steps involved in the synthesis and replacement of RNA primers during the discontinuous replication of the lagging strand are illustrated in ■ Figure 10.18.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 236

Termination step:

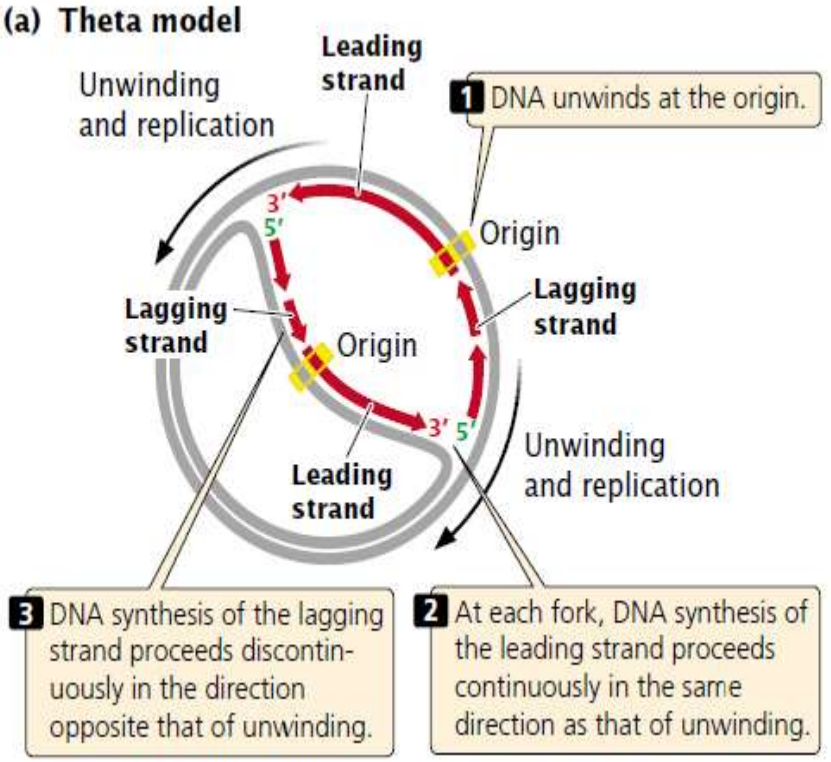
- In bacterial genome, replication is bidirectional from single point
- 2 replication fork should meet at a position diametrically opposite the origin of replication
- Replication terminates at terminus region

Terminus region:

- Contains multiple copies of about 23 bp sequences called **Ter** (for **terminus**)
- Each acting as recognition site for a sequence specific DNA binding protein called **Tus (terminus utilization substance)**

Tus protein+ Ter sequence

- Allows replication fork to pass if fork is moving ion one direction
- Blocks progress if fork is moving in opposite direction around genome
- Directionality is set by orientation of Tus protein on double helix
- When approached from one direction- Tus blocks the passage of **DnaB Helicase**
- When approached from other direction- DnaB –able to cross **Tus protein**


(a) Theta model
Leading strand
Unwinding and replication
1 DNA unwinds at the origin.
3′
5′
Origin
Lagging strand
Lagging strand
Origin
3′
5′
Unwinding and replication
Leading strand
3 DNA synthesis of the lagging strand proceeds discontinuously in the direction opposite that of unwinding.
2 At each fork, DNA synthesis of the leading strand proceeds continuously in the same direction as that of unwinding.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 332

Completion of replication in circular chromosome,

2 new circles may be physically interlocked/ catenated

Separated so that each daughter cell receives single dsDNA upon cell division

Decatenation of interlocked circle- By **Topoisomerase IV**

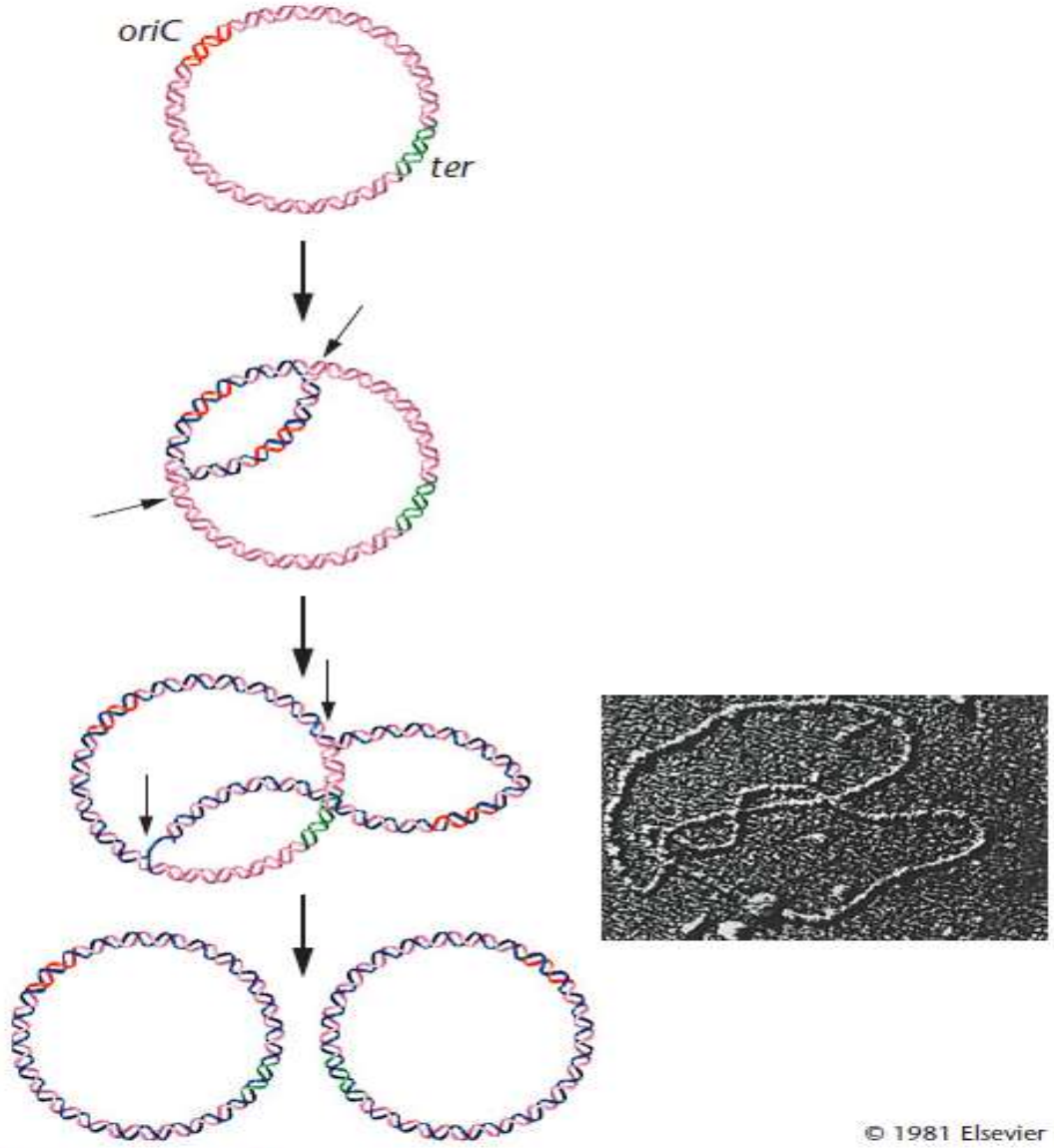


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FIGURE 11–6 Bidirectional replication of the *E. coli* chromosome. The thin black arrows identify the advancing replication forks. The micrograph is of a bacterial chromosome in the process of replication, comparable to the figure next to it.

Source: Klug cummins, Spencer, Palladino (2012), Concepts of Genetics, Pearson Publication, 10th Edition, Page no.274

Rashmi M G

DNA replication in Eukaryotes

To summarize,
Steps involved in DNA replication in Prokaryotes

1. DnaA protein+ oriC (9 mer)- Initial complex (ATP NEEDED)
2. DnaB protein- Helicase- for further melting
3. DnaC- Helicase loader- joins initial complex
4. SSB protein – Keeps unwound DNA strand in extended form
5. RNA primase- DnaG- For primer synthesis
6. DNA polymerase- Initiation of DNA polymerization
7. DNA polymerase I- Removal of primer
8. DNA polymerase II- DNA repair activity
9. DNA polymerase III- Main replicating enzyme- chain elongation, proof reading
10. DNA ligase- Joining of fragments (ATP NEEDED)

The eukaryotic DNA replication is similar to prokaryotic DNA replication but differences lies in these following aspects,

1. Multiple replication origins in their chromosomes
2. More types of DNA polymerases with different functions
3. Linear DNA replication
4. Nucleosome assembly immediately after DNA replication
5. Different termination strategy

Unique aspects of eukaryotic DNA replication

1. **Multiple replication origins**

- Eukaryotic genome= greater amount of DNA
- Ex. Yeast, *Drosophila*
- Replication takes more time
- To facilitate the rapid synthesis of large quantities of DNA, Eukaryotic chromosomes contain multiple replication origins
- Origins in yeast- Autonomously replication sequences
- Eukaryotic replication origins- not only acts as sites of replication initiation, but also control the timing of DNA replication
- These regulatory functions are carried out by a complex of more than 20 proteins, called Pre-replication complex (Pre-RC), which assembles at replication origins.

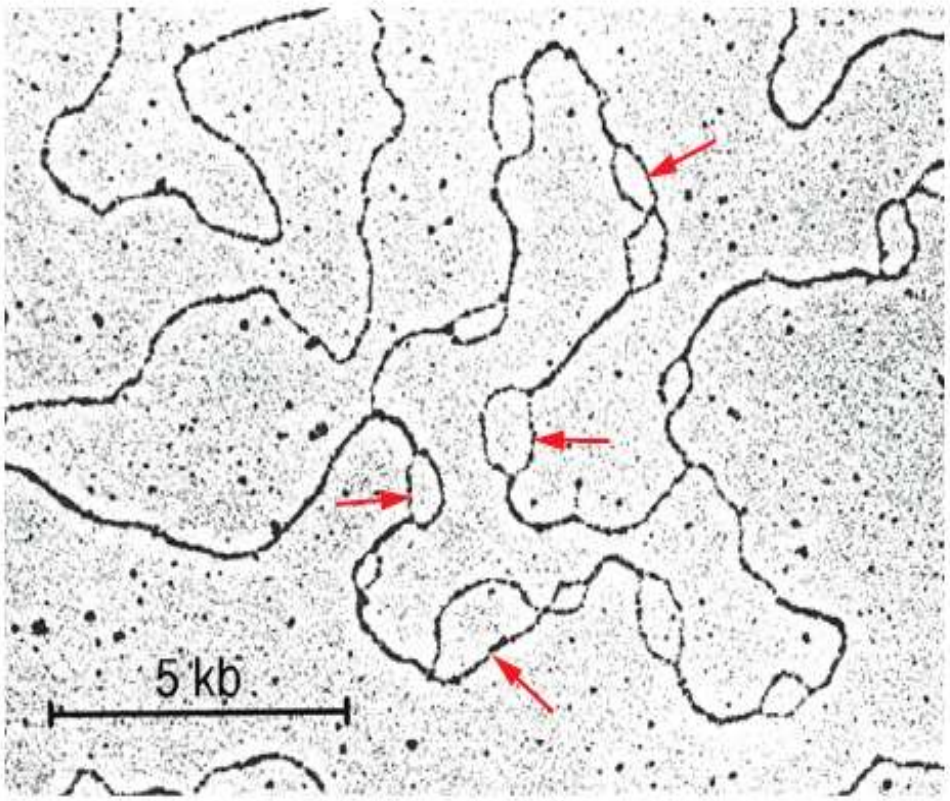


FIGURE 11–14 A demonstration of the multiple origins of replication along a eukaryotic chromosome. Each origin is apparent as a replication bubble along the axis of the chromosome. Arrows identify some of these replication bubbles.

Source: Klug cummins, Spencer, Palladino (2012), Concepts of Genetics, Pearson Publication, 10th Edition, Page no.282

2. More types of DNA polymerases with different functions

Eukaryotic cell contains,

Many types of DNA polymerase

Present both in nucleus and organelles (Mitochondria and chloroplast)

3 major types of DNA polymerase (present in nucleus for nuclear DNA replication):

1. **DNA polymerase α**
2. **DNA Polymerase δ**
3. **DNA polymerase ϵ**

Enzyme	Exonuclease activity		
	3'→5'	5'→3'	Function
DNA polymerase α	NO	NO	Priming during replication
DNA polymerase β	NO	NO	Base excision repair
DNA polymerase γ	YES	NO	Mitochondrial DNA replication
DNA polymerase δ	YES	NO	Lagging and leading strand synthesis
DNA polymerase ϵ	YES	NO	-

Major DNA polymerase involved in Replication of Eukaryotic genomes

Eukaryotic DNA polymerases

1. DNA polymerase α

- Unusual polymerase
- Has both DNA polymerase and primase activity
- 5'→3' DNA dependent DNA polymerase activity
- 5'→3' DNA dependent RNA polymerase activity
- Heterotetramer

Primase activity:

- Incorporates NMP- To synthesize short RNA primer (iRNA initiator RNA)
- Complement – ssDNA template

Polymerase activity:

- Adds 20-30 NMP to 3' end of iRNA (also DNA – iDNA)
- No proofreading activity
- (absence of 3'→ 5' exonuclease activity

Function:

- Initiates DNA synthesis on both leading and lagging strand
- Polymerase α + initiation complex- at origin:
- Synthesize short iRNA followed by 20-30 bases of iDNA
- Then replaced by other DNA polymerase- extend the chain
- This process is called- **polymerase switching**

2. DNA Polymerase δ

- Multi subunit nuclear enzyme
- 4 subunits
- Has 5'→3' polymerase activity
- 3'→5' exonuclease activity
- Requires associated 30kDa-protein (proliferating cell nuclear antigen PCNA) function as sliding clamp
- For high Processivity
- Requires clamp loader- replication factor C acts as clamp loader

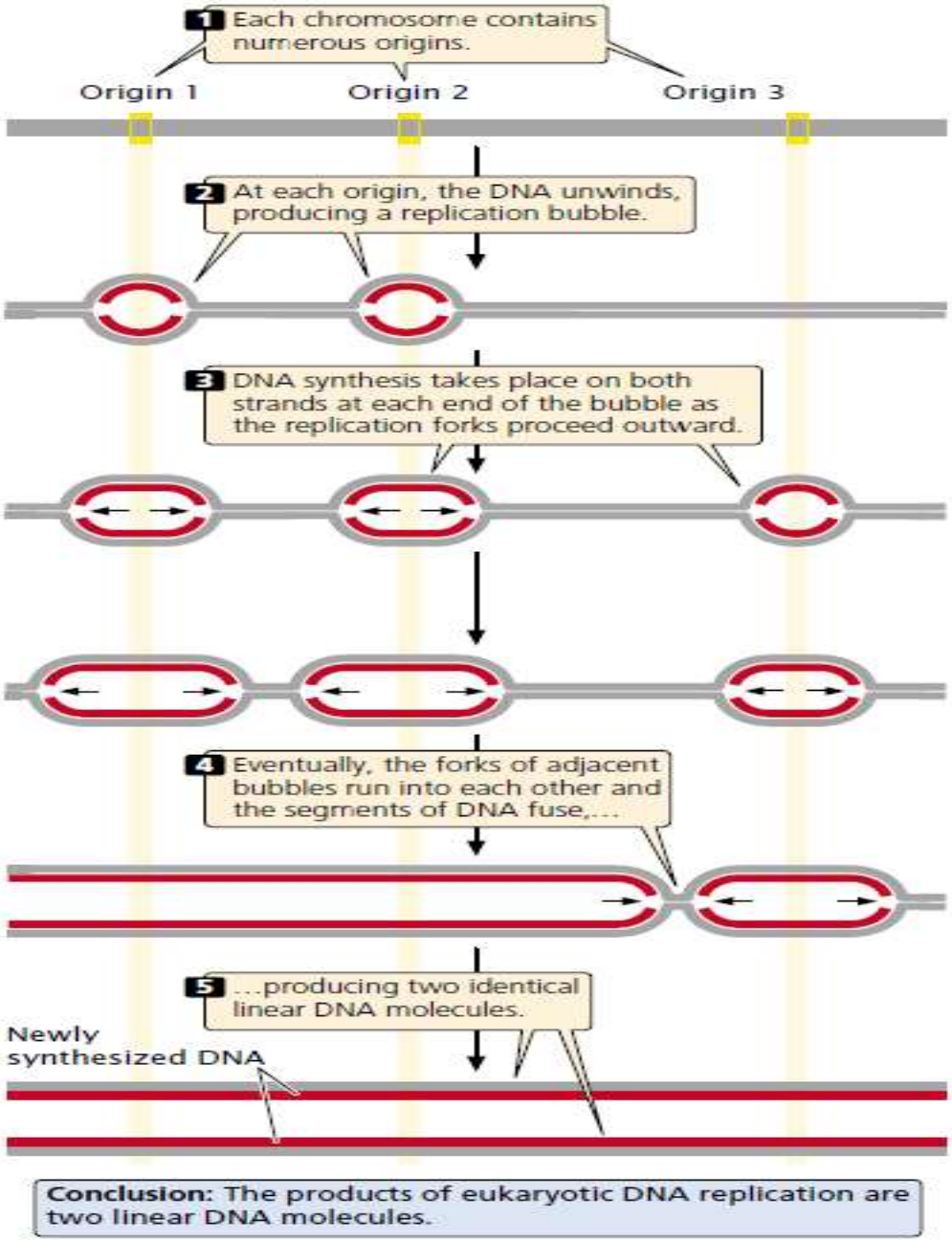
Function: Alone replicates leading and lagging strands of DNA

3. DNA polymerase ϵ

- Multi subunit nuclear enzyme
- 4 subunits
- 5'→3' polymerase activity
- 3'→ 5' exonuclease activity
- High processive DNA synthesis with help of sliding clamp and clamp loader
- **Function**: Not known precisely

4. DNA polymerase γ

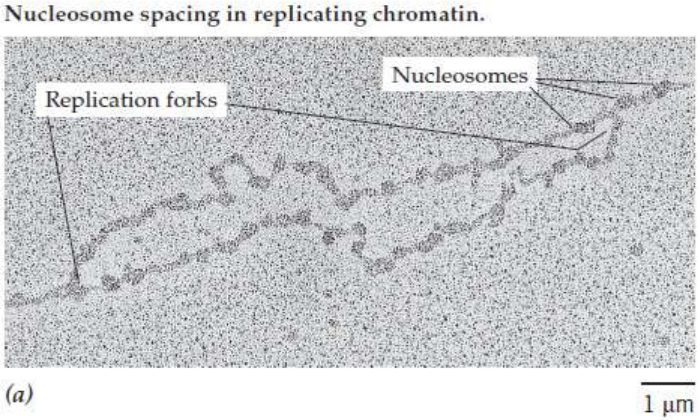
- Sole polymerase
- Participate in mitochondrial DNA replication
- 3 subunits
- 5'→3' polymerase activity
- 3'→5' exonuclease activity

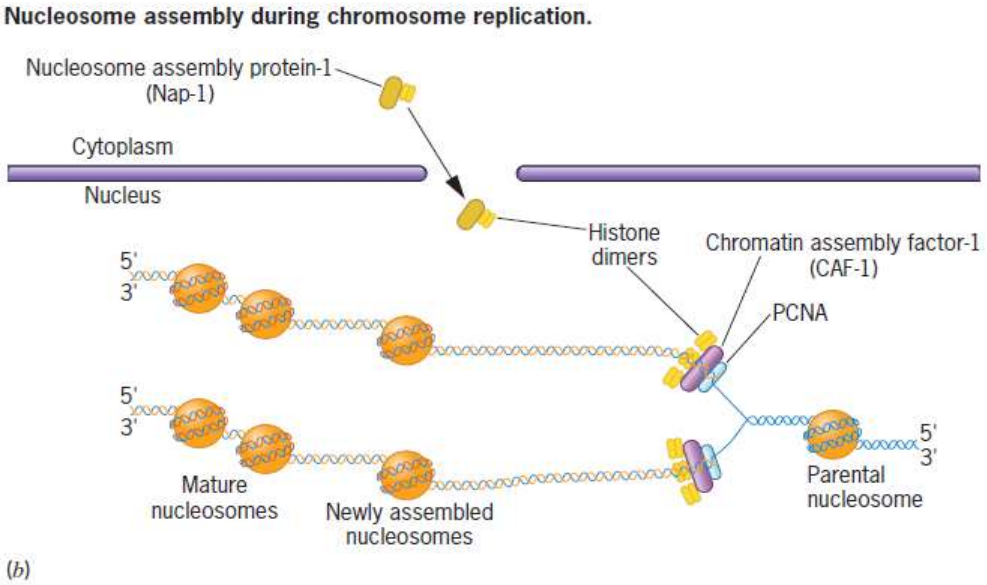

1 Each chromosome contains numerous origins.
Origin 1
Origin 2
Origin 3
2 At each origin, the DNA unwinds, producing a replication bubble.
3 DNA synthesis takes place on both strands at each end of the bubble as the replication forks proceed outward.
4 Eventually, the forks of adjacent bubbles run into each other and the segments of DNA fuse,...
5 ...producing two identical linear DNA molecules.
Newly synthesized DNA
Conclusion: The products of eukaryotic DNA replication are two linear DNA molecules.

12.6 Linear DNA replication takes place in eukaryotic chromosomes.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 329

3. Nucleosome assembly immediately after DNA replication





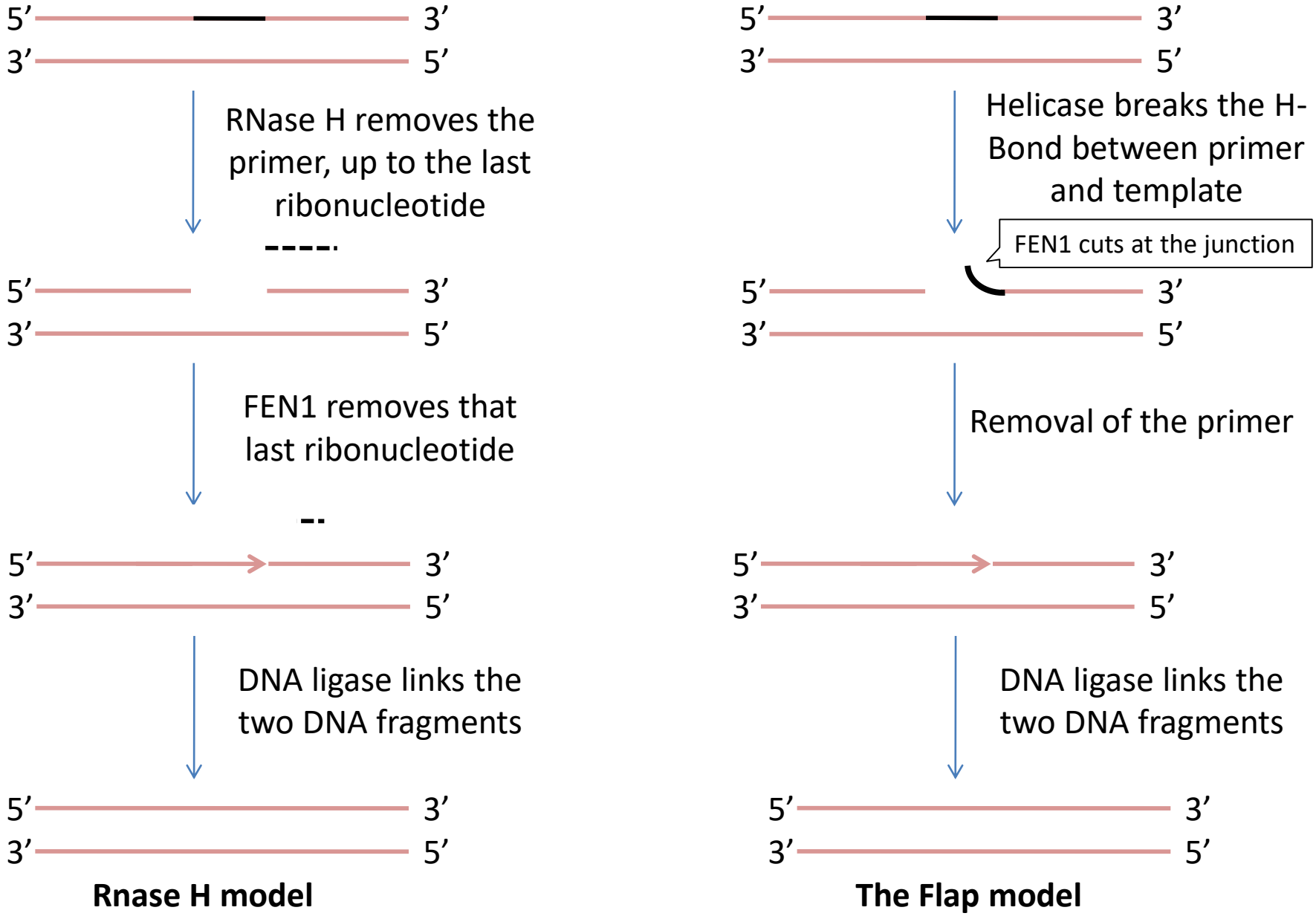
■ **FIGURE 10.33** The disassembly and assembly of nucleosomes during the replication of chromosomes in eukaryotes. (*a*) An electron micrograph showing nucleosomes on both sides of two replication forks in *Drosophila*. Recall that DNA replication is bidirectional; thus, each branch point is a replication fork. (*b*) The assembly of new nucleosomes during chromosome replication requires proteins that transport histones from the cytoplasm to the nucleus and that concentrate them at the site of nucleosome assembly. PCNA = proliferating cell nuclear antigen (see Figure 10.32).

- Eukaryotic DNA is complexed to histone proteins in nucleosome structures that contribute stability and packing of the DNA molecule.
- The disassembly and reassembly of nucleosomes on newly synthesized DNA probably takes place in replication, but the precise mechanism for these processes has not yet been determined
- Before replication, a single DNA molecule is associated with histone proteins
- After replication and nucleosome assembly, two DNA molecules are associated with histone proteins

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 247

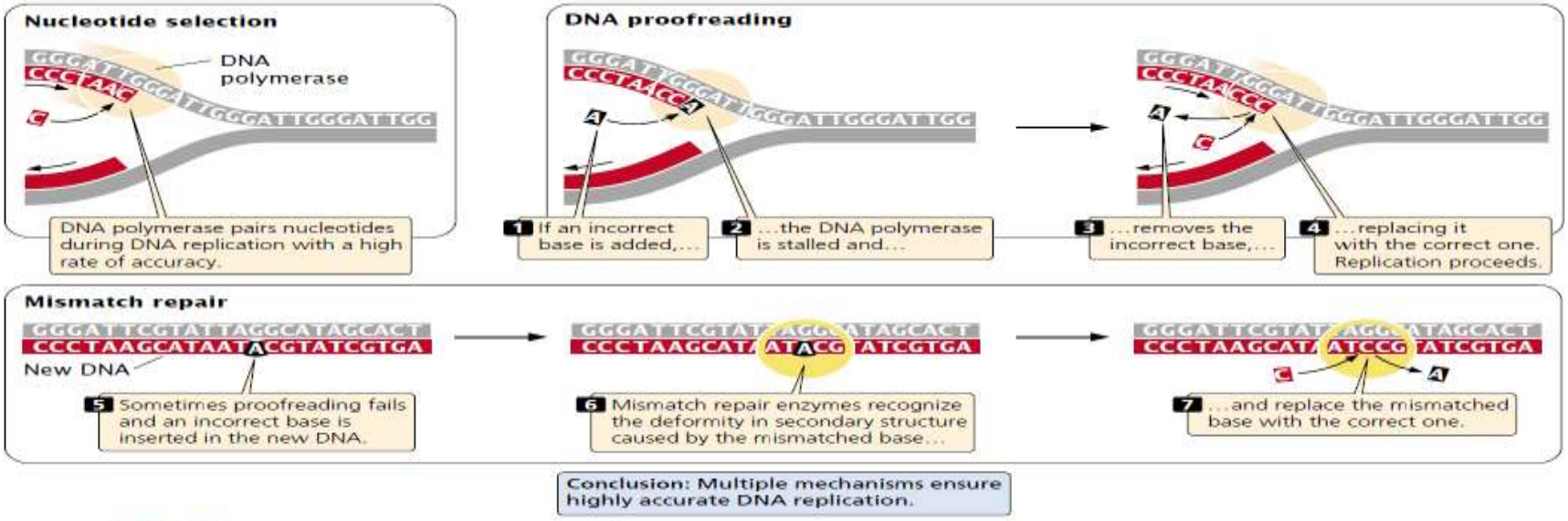
Rashmi M G

4. Termination step in Eukaryotic replication


5’
3’
3’
5’
RNase H removes the primer, up to the last ribonucleotide
FEN1 removes that last ribonucleotide
DNA ligase links the two DNA fragments
Rnase H model
Helicase breaks the H-Bond between primer and template
FEN1 cuts at the junction
Removal of the primer
DNA ligase links the two DNA fragments
The Flap model

Proofreading

- Needed by mammals (in large genomes)
- Scanning the termini of nascent DNA chain for errors
- Correcting them before continuing chain extension
- Carried out by 5'→3' exonuclease activity that is built into DNA polymerases
- When template- primer DNA has a terminal mismatch (an unpaired/ incorrectly paired base/ sequence of bases at 3' of primer)
- 3'→5' exonuclease activity of DNA polymerase I or II hips off the unpaired base/ bases
- When appropriate base- paired terminus is produced
- The 5'→3' polymerase activity of enzyme begins resynthesis by adding nucleotides to 3' end of primer strand


Nucleotide selection
DNA polymerase
DNA polymerase pairs nucleotides during DNA replication with a high rate of accuracy.
DNA proofreading
1 If an incorrect base is added,...
2 ...the DNA polymerase is stalled and...
3 ...removes the incorrect base,...
4 ...replacing it with the correct one. Replication proceeds.
Mismatch repair
New DNA
5 Sometimes proofreading fails and an incorrect base is inserted in the new DNA.
6 Mismatch repair enzymes recognize the deformity in secondary structure caused by the mismatched base...
7 ...and replace the mismatched base with the correct one.
Conclusion: Multiple mechanisms ensure highly accurate DNA replication.

12.16 A series of processes are required to ensure the incredible accuracy of DNA replication. Among these processes are DNA selection, proofreading, and mismatch repair.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 339

Comparisons

Enzyme/ Protein	*E coli*	Human
Helicase	DnaB	MCM
ssDNA binding protein	SSB	RPA (Replication protein A)
Primase	DnaG	Dna polymeraseα/ Primase
Replicase	DNA polymerase III	DNA polymerase ϵ / DNA polymerase δ
Topoisomerase	Gyrase	Topo I, II
Processivity component	β clamp	PCNA (Proliferating cell nuclear antigen)
Clamp loader	γ-complex	RFC (Replication factor C)

DNA replicating enzymes/ proteins from *E coli* and Human

Quantitative parameters	*E coli*	Human
DNA content, number of nucleotide pairs per cell	3.9×10^6	About 10^9
Rate of replication fork progression per replication (per second)	~1000	~100
Number of replication origins per cell	1	10^3-10^4
Time required for complete genome replication	~42 minutes	~8hours

Comparison of quantitative parameters of DNA replication in *E coli* and Human